MESOTRIONE

First draft prepared by
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Explanation

Mesotrione is the International Organization for Standardization (ISO)–approved common name for 2-(4-mesyl-2-nitrobenzoyl)cyclohexane-1,3-dione (International Union of Pure and Applied Chemistry), with Chemical Abstracts Service number 104206-82-8. It is a new triketone herbicide with a 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibition mode of action.

Mesotrione has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and was reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues.

All critical studies contained statements of compliance with good laboratory practice (GLP) or good clinical practice and the Declaration of Helsinki, as appropriate.

Evaluation for acceptable daily intake

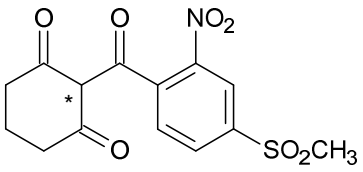
1. Biochemical aspects

The absorption, distribution, metabolism and excretion, as well as the toxicokinetics, of mesotrione have been investigated in CD-1:Crl(ICR)BR mice and Alpk:APfSD rats. Summaries of the relevant data are presented below.

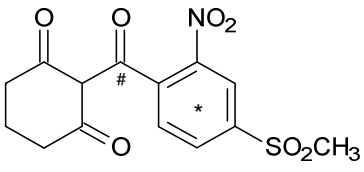
1.1 Absorption, distribution and excretion

The absorption, distribution, metabolism and excretion (ADME) of mesotrione in CD-1:Crl(ICR)BR mice and Alpk:APfSD rats were first investigated using the active substance radiolabelled in the cyclohexane and phenyl rings (Fig. 1). Upon confirmation in biliary elimination and biotransformation studies that there were no pronounced differences in the metabolism of the two labelled forms, subsequent ADME studies were performed with [^{14}C-aromatic]mesotrione (Gledhill, 1996). This finding was confirmed in the qualitative whole-body autoradiographic study in the rat, in that there were no clear differences in tissue distribution profiles between [^{14}C-dione]mesotrione and [^{14}C-aromatic]mesotrione (Prescott & Bennet, 1995). The test item was a mixture of ^{14}C-labelled mesotrione (phenyl or dione label; Fig. 1) and unlabelled mesotrione. Radiolabelled mesotrione was administered via oral gavage in a water/sodium bicarbonate vehicle. The study design is summarized in Table 1.

Fig. 1. Structure of mesotrione with radiolabel positions for the ADME studies



(* = ^{14}C position)



(* = ^{14}C position)
(# = The exocyclic carbonyl group was enriched with ^{13}C for some studies)

Source: Gledhill (1996)

The data generated with mesotrione (including intravenous dosing) indicated that mesotrione is rapidly and extensively absorbed, with almost complete bioavailability.

In mice, total recovery of radioactivity at 72 hours following oral administration ranged from 90.76% to 95.06% of the administered dose, with the main route of excretion being the urine in males and females at 1 or 100 mg/kg body weight (bw) (Table 2). In low-dose groups, the majority of excretion was in the first 6 hours. In high-dose groups, the majority was excreted in the urine in hours 0–6; however, a significant amount of radioactivity was excreted between hours 6 and 12. The majority of radioactivity excreted in the faeces in mice was excreted in the first 12 hours. In the low-dose groups, 14–15% of the administered dose was recovered in the tissues and carcass. In the high-dose groups, less than 0.5% of the administered dose was recovered in the tissues and carcass. Mesotrione was therefore extensively absorbed (40–70% of the administered dose) and rapidly excreted following oral administration.

Following termination at 72 hours, the carcass and select tissues of the animals were examined. Other than the amounts in the gastrointestinal tract and carcass (Table 2), residual radioactivity was found in the liver (13–14% in the low-dose groups and 0.2–0.3% in the high-dose groups) and kidney (0.2% and 1.0% in low-dose males and females, respectively, and 0.003% and 0.015% in high-dose males and females, respectively). Trace amounts of residual radioactivity (< 0.005%) were found in the brain, gonads, heart, lungs and spleen (Gledhill, 1997).

Table 1. Dosing groups for balance/excretion experiments with [^{14}C]mesotrione

Test group	Dose of labelled material (mg/kg bw)	Number of animals of each sex	Remarks	Reference
Single oral low dose in the mouse – Excretion and tissue retention	1	4 mice	A single nominal dose of 1 mg/kg bw (4 mL of 0.25 mg/kg bw) of [^{14}C-aromatic]mesotrione (specific activity for 4 mL: 4 MBq/kg) was administered by oral gavage. Killed after 72 h.	Gledhill (1997)
Single oral high dose in the mouse – Excretion and tissue retention	100	4 mice	A single nominal dose of 100 mg/kg bw (4 mL of 25.4 mg/kg bw) of [^{14}C-aromatic]mesotrione (specific activity for 4 mL: 8 MBq/kg) was administered by oral gavage. Killed after 72 h.	Gledhill (1997)
Single oral low dose in the rat – Excretion and tissue retention	1	5 rats	A single nominal dose of 1 mg/kg bw (4 mL of 0.25 mg/kg bw) of [^{14}C-aromatic]mesotrione (specific activity for 4 mL: 4 MBq/kg) was administered by oral gavage. Killed after 72 h.	Macpherson (1996a)
Single intravenous low dose in the rat – Excretion and tissue retention	1	8 rats	A single nominal dose of 1 mg/kg bw (4 mL of 0.22 mg/kg bw) of [^{14}C-aromatic]mesotrione (specific activity for 4 mL: 4.185 MBq/kg) was administered by injection. Killed after 72 h.	Macpherson (1996b)
Repeated oral low dose in the rat	1	8 rats	*Unlabelled*: Fourteen daily doses of 1 mg/kg bw (4 mL of 0.25 mg/mL) of mesotrione. *Labelled*: A single nominal dose of 1 mg/kg bw (4 mL of 0.26 mg/kg bw) of [^{14}C-aromatic]mesotrione (specific activity for 4 mL: 4.185 MBq/kg) was administered by oral gavage. Killed after 72 h.	Macpherson (1996c)
Single oral high dose in the rat	100	5 rats	A single nominal dose of 100 mg/kg bw (4 mL of 24.94 mg/kg bw) of [^{14}C-aromatic]mesotrione (specific activity for 4 mL: 6 MBq/kg) was administered by oral gavage. Killed after 72 h.	Macpherson (1996d)
Whole-body autoradiography in the rat	5	2 rats per radiolabel	A single nominal dose of 5 mg/kg bw (4 mL/kg bw) of [^{14}C-dione]mesotrione (specific activity for 4 mL/kg bw: 6 MBq/kg) was administered by oral gavage. Killed at 24 and 48 h after dosing. A single nominal dose of 5 mg/kg bw (4.25 mL/kg bw) of [^{14}C-aromatic]mesotrione (specific activity for 4.25 mL/kg bw: 6 MBq/kg) was administered by oral gavage. Killed at 24 and 48 h after dosing.	Prescott & Bennet (1995)
Biotransformation in the rat	50 [^{14}C-dione]-mesotrione 50 or 100 [^{14}C-aromatic]-mesotrione	2 rats per dose per radiolabel	A single nominal dose of 50 mg/kg bw (4 mL/kg bw) of [^{14}C-dione]mesotrione (specific activity of dosing solution 4.15 MBq/g) was administered by oral gavage. A single nominal dose of 50 mg/kg bw (4 mL/kg bw) of [^{14}C-aromatic]mesotrione (specific activity of dosing solution 1.59 MBq/g) was administered by oral gavage. A single nominal dose of 100 mg/kg bw (4 mL/kg bw) of [^{14}C-aromatic]mesotrione (specific activity of dosing solution 3.61 MBq/g) was administered by oral gavage.	Gledhill (1996)

Table 1 (continued)

Test group	Dose of labelled material (mg/kg bw)	Number of animals of each sex	Remarks	Reference
Single oral low dose in the rat – Excretion and distribution	1	4 rats	A single nominal dose of 1 mg/kg bw (4 mL/kg bw) of [^{14}C-aromatic]mesotrione (specific activity of dosing solution 4.13 MBq/kg) was administered by oral gavage. Killed after 7 days.	Duerden (2005a)
Single oral low dose in the rat – Pharmacokinetics and tissue depletion	1	9 rats per phase	A single nominal dose of 1 mg/kg bw (4 mL/kg bw) of [^{14}C-aromatic]mesotrione (specific activity of dosing solution 4.13 MBq/kg) was administered by oral gavage. Serial blood collection at 0.5, 1.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24, 30, 48, 72 and 96 h. A single nominal dose of 1 mg/kg bw (4 mL/kg bw) of [^{14}C-aromatic]mesotrione (specific activity of dosing solution 4.13 MBq/kg) was administered by oral gavage. Killed at 1, 6, 12, 24, 48 and 96 h.	Duerden (2005b)
Single oral low dose in the rat – Pharmacokinetics and tissue depletion	100	9 rats per phase	A single nominal dose of 1 mg/kg bw (4 mL/kg bw) of [^{14}C-aromatic]mesotrione (specific activity of dosing solution 4.13 MBq/kg) was administered by oral gavage. Serial blood collection at 0.5, 1.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24, 30, 48, 72 and 96 h. A single nominal dose of 1 mg/kg bw (4 mL/kg bw) of [^{14}C-aromatic]mesotrione (specific activity of dosing solution 4.13 MBq/kg) was administered by oral gavage. Killed at 1, 6, 12, 24, 48 and 96 h.	Duerden (2005b)

bw: body weight

In rats, total recovery of radioactivity at 72 hours ranged from 92.02% to 97.06% of the administered dose (Table 3). In all dose groups, excretion in the urine comprised over 50% of the administered dose. Excretion in the faeces constituted 23–30% of the administered dose in orally dosed groups and 2–7% in the intravenously dosed group, indicating that there is a significant amount of the administered dose excreted unabsorbed and that bile excretion is not a major route of excretion in the rat. However, oral absorption was greater than 60% in all dose groups. Residual radioactivity in the tissues and carcass comprised 11–12% in the oral low-dose group, 5% in the oral repeated-dose group and 10% in the intravenous low-dose group. In the oral high-dose group, residual radioactivity in the tissues and carcass comprised 0.7–1% of the administered dose. Results of the single oral low-dose study were confirmed in a second study (Duerden, 2005a), which extended the recovery period to 168 hours (Macpherson, 1996a,b,c,d).

Following termination at 72 hours, the carcass and select tissues of the animals were examined. Other than residual radioactivity in the gastrointestinal tract (0.009–0.031% of the administered dose) and carcass (0.180–1.353% of the administered dose), the only quantifiable residual radioactivity was found in the kidney and liver. In the single oral and intravenous low-dose groups, 9–12% of the administered radioactivity was found in the liver. In the same groups, 0.3% and 0.8–0.9% of the administered dose were found in the kidneys of males and females, respectively. In the single oral high-dose group, 0.2% of the administered dose was found in the liver and 0.01% in the kidneys. In the repeated low-dose group, 4% of the administered dose was found in the liver of males and females and 0.1% and 0.4% in the kidneys of males and females, respectively. In all dose groups, radioactivity

Table 2. Excretion balance in mice at 72 hours post-dosing

	% of administered dose			
	Single oral low dose (1 mg/kg bw)		Single oral high dose (100 mg/kg bw)	
Route and time (h)	**Males**	**Females**	**Males**	**Females**
Urine				
0–6	31.35 ±24.21	51.08 ± 6.64	44.54 ± 17.63	42.68 ± 9.21
6–12	3.07 ± 1.62	4.40 ± 2.27	13.38 ± 5.91	22.20 ± 9.03
12–24	2.55 ± 1.76	1.74 ± 0.64	1.09 ± 0.08	3.37 ± 1.69
24–36	2.23 ± 1.98	0.67 ± 0.34	2.16 ± 2.09	0.99 ± 0.37
36–48	0.86 ± 0.82	0.44 ± 0.14	1.60 ± 2.16	0.39 ± 0.05
48–72	0.56 ± 0.17	0.28 ± 0.20	0.14 ± 0.11	0.08 ± 0.01
Subtotal	**40.64**	**58.61**	**62.90**	**69.82**
Faeces				
0–12	24.88 ± 2.14	16.63 ± 3.37	22.01 ± 2.72	18.06 ± 1.53
12–24	5.41 ± 4.44	2.61 ± 1.44	1.81 ± 1.39	4.53 ± 1.04
24–36	2.60 ± 2.29	0.84 ± 0.24	0.57 ± 0.54	0.98 ± 0.33
36–48	0.63 ± 0.57	0.22 ± 0.03	2.48 ± 3.38	0.62 ± 0.16
48–72	4.13 ± 3.82	0.58 ± 0.15	0.40 ± 0.33	0.28 ± 0.01
Subtotal	**37.66**	**20.88**	**27.27**	**24.46**
Tissues	13.94	13.84	0.18	0.28
Carcass	0.842	0.300	0.096	0.126
Cage wash	0.62	0.90	0.31	0.37
Total	**93.70**	**94.53**	**90.76**	**95.06**

bw: body weight

Source: Gledhill (1997)

in the brain, gonads, heart, lungs or spleen was below 0.001% of the administered dose (Macpherson, 1996a,b,c,d).

Whole-body autoradiography confirmed that the major route of excretion was the urine and that both the kidneys and the liver were subject to tagging by the radiolabelled compounds or their respective metabolites. The gastrointestinal tract and contents appeared to contain the greatest amounts of radiolabelled compound, which would be expected in association with faecal elimination (Prescott & Bennet, 1995).

Pharmacokinetics was generally similar between low and high doses and between sexes. The peak plasma concentrations (C_{max}) were 0.27 and 0.25 µg equivalents (eq) per gram in the low-dose groups of males and females, respectively. In the high-dose groups, the C_{max} values were 40.4 µg eq/g in males and 19.9 µg eq/g in females. The times to reach C_{max} (T_{max}) were 0.5 hour in the low-dose groups and 1.5 hours in the high-dose groups. The half-lives in blood were 1.6 and 1.4 hours in low-dose males and females, respectively, and 1.7 and 1.8 hours in high-dose males and females, respectively (Duerden, 2005b).

Table 3. Recovery of radioactivity in tissues and excreta of rats

	% of administered dose							
	Single low dose[a]		Single high dose[b]		Intravenous dose[c]		Repeated low dose[d]	
	Males	Females	Males	Females	Males	Females	Males	Females
Expired air	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Tissues	12.21	10.17	0.26	0.21	10.01	9.85	4.58	3.98
Carcass	0.25	1.07	0.44	0.90	0.33	0.18	0.53	1.35
Cage wash	0.90	1.36	0.73	0.97	0.46	0.53	0.24	0.76
Urine	54.15	55.88	61.54	63.02	79.40	84.14	60.84	66.97
Faeces	24.51	23.80	30.49	28.77	6.76	2.36	30.27	23.11
Total	**92.02**	**92.28**	**93.46**	**93.87**	**96.96**	**97.06**	**96.46**	**96.17**

N/A: not available

[a] *Source*: Macpherson (1996a).
[b] *Source*: Macpherson (1996d).
[c] *Source*: Macpherson (1996b).
[d] *Source*: Macpherson (1996c).

1.2 Biotransformation

The urinary and faecal samples from the Gledhill (1997) study were subjected to high-performance liquid chromatographic (HPLC) analysis to determine the metabolic fate of mesotrione. The vast majority of mesotrione was excreted as unchanged parent via the urine (43–64%), with 0–8% excreted in the faeces. Proportionately, there was a greater amount of unknown metabolites in the faeces, indicating metabolism of mesotrione by the intestinal flora (0–12% of the administered dose). The chemical structures and retention times of metabolites are provided in Table 4 (Gledhill, 1996).

The proposed metabolic pathway in the rat is shown in Fig. 2 and proceeds by either hydroxylation of the dione ring or, following biliary excretion of unchanged mesotrione, by cleavage of the molecule into its two constituent rings and reduction by the gut microflora (Gledhill, 1996).

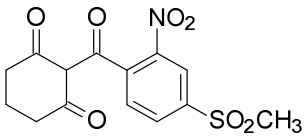
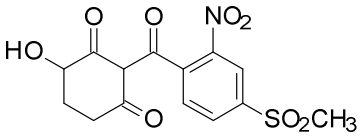
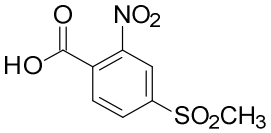
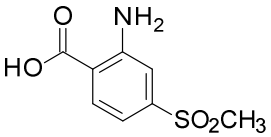
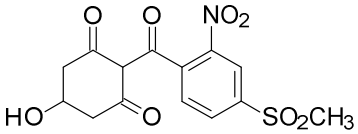
2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

Mesotrione is of low acute toxicity in rats via the oral, dermal and inhalation routes (Table 5). All tests were performed at the limit dose. Clinical signs in the oral toxicity study were limited to staining around the nose and lack of body weight gain in females from days 3 to 8. In the dermal toxicity study, staining about the nose was noted in three males, and salivation and urinary incontinence were noted in 3–4 females. Signs of irritation, scabbing, desquamation, oedema and yellow staining were noted at the test site. There were no changes noted at gross necropsy in the oral or dermal studies, and all animals in the dermal toxicity study gained weight throughout the study period. In the inhalation toxicity study, clinical signs of toxicity consisted of hunched posture, piloerection, irregular breathing and/or abnormal respiratory noise, salivation, wet fur, decreased activity, head and paw flicking, decreased response to sound, shaking, reduced righting reflex, mucous secretion from the nose, reduced stability, ptosis, absence of pinna reflex, stains around the nose, splayed gait and chromodacryorrhoea. All animals gained weight following day 2. Pinpoint red spots were noted in the lungs of one male (Robinson, 1994a,b; Rattray, 1995).

Table 4. Chemical structures of identified metabolites

Metabolite designation (code)	Retention time (min)	Structure	Urine	Faeces
Mesotrione	21		+	+
4-Hydroxy mesotrione	15		+	+
MNBA (2-nitro-4-(methylsulfonyl)-benzoic acid)	6		+	+
AMBA (4-(methylsulfonyl)-2-aminobenzoic acid)	9		+	+
5-Hydroxy mesotrione	–		+	+

–: not given

Source: Gledhill (1996)

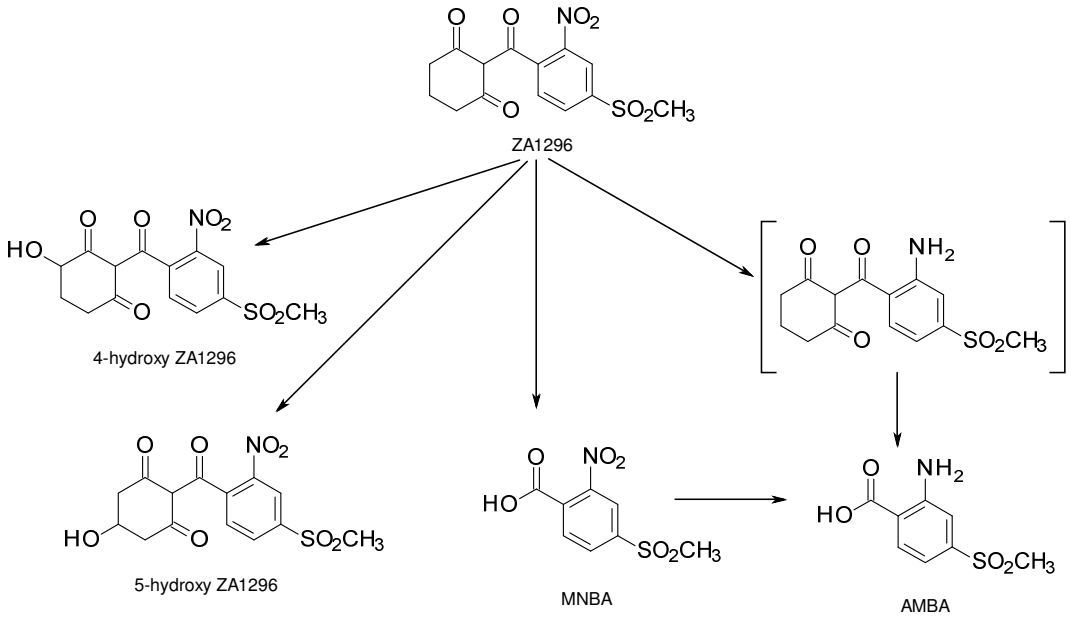
(b) Ocular irritation

In an eye irritation study, 0.1 g mesotrione (purity 95.1%; batch no. WRC4845-32-2) was applied into the conjunctival sac of the left eye of nine female New Zealand White rabbits. In six animals, the eyes were left unwashed, and in a subsequent group of three animals, the eyes were washed immediately following instillation. Animals were then observed for 3 or 4 days. Irritation was scored by the method of Draize. In the non-irrigation study, mild corneal opacity and iritis were noted at 1 hour following instillation, and mild to moderate conjunctivitis was noted from hours 1 to 72. In the irrigation study, there were no signs of corneal opacity or iritis, and mild to moderate conjunctivitis was noted from hours 1 to 48. Mesotrione would be considered mildly irritating to the eyes of rabbit due to continued irritation at 72 hours in the non-irrigation study (Robinson, 1994c).

(c) Dermal irritation

In a dermal irritation study, six adult male New Zealand White rabbits were exposed to 0.5 g mesotrione (purity 95.1%; batch no. WRC14845-32-2) applied to 6.25 cm^2 of body surface area with occlusion for 4 hours. Following exposure, the test site was washed with tap water, and the animals

Fig. 2. Proposed metabolic pathway of mesotrione in rat



Source: Gledhill (1996)

Table 5. Acute toxicity of mesotrione

Species	Strain	Sex	Route	Purity (%)	Result	Reference
Rat	Alpk:APfSD (Wistar-derived)	Male and female	Oral	95.1	LD_{50} > 5 000 mg/kg bw	Robinson (1994a)
Rat	Alpk:APfSD (Wistar-derived)	Male and female	Dermal	95.1	LD_{50} > 2 000 mg/kg bw	Robinson (1994b)
Rat	Alpk:APfSD (Wistar-derived)	Male and female	Inhalation	97.3	LC_{50} > 4.75 mg/L	Rattray (1995)

bw: body weight; LC_{50}: median lethal concentration; LD_{50}: median lethal dose

were observed for 3 days. At 1 hour, 2/6 animals exhibited slight erythema and oedema, which cleared by 24 hours. No oedema was observed (Robinson, 1994d).

(d) Dermal sensitization

The skin sensitization potential of mesotrione (purity 95.1%; batch no. WRC14845-32-2) was investigated using the guinea-pig maximization test. Twenty female albino guinea-pigs were assigned to the test group, and an additional 20 female albino guinea-pigs were assigned to the vehicle control group. Test animals were treated with three injections consisting of Freund's Complete Adjuvant plus deionized water (1:1), a 3% weight per volume (w/v) preparation of test material in deionized water and a 3% w/v preparation of test material in a 1:1 preparation of Freund's Complete Adjuvant plus deionized water. Vehicle control animals were treated with three injections of Freund's Complete Adjuvant plus deionized water in a 1:1 ratio, deionized water and Freund's Complete Adjuvant plus deionized water in a 1:1 ratio. The following week, 0.3 g of test substance in a 75% w/v preparation in deionized water for test animals or deionized water for vehicle control animals was applied to an area of the scapular region clipped free of fur. The preparation was kept under occlusive dressing for 2 days. Two weeks following the topical induction application, preparations of 75% w/v mesotrione in

deionized water and 30% w/v mesotrione in deionized water were applied to clipped skin of the left flank of both test and vehicle control animals, and two gauze patches containing only deionized water were applied to the clipped skin of the right flank for 24 hours under occlusive dressing. Irritation was then scored according to Draize.

No irritation was noted in the test animals during the induction phase, and no irritation was noted in the test or vehicle control group following the challenge application. The response in the positive control animals validated the test. It was concluded that mesotrione does not have a sensitizing effect on the skin in the guinea-pig maximization test (Robinson, 1994e).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 90-day study, groups of 20 (40 control) C57BL/10JfCD-1 mice of each sex per dose received mesotrione (purity 96.8%) in the diet at a dose level of 0, 10, 50, 350 or 7000 parts per million (ppm) (equal to 0, 1.7, 8.4, 61.5 and 1212.4 mg/kg bw per day for males and 0, 2.4, 12.4, 80.1 and 1537.1 mg/kg bw per day for females, respectively). Animals were observed daily for morbidity, moribundity and overt clinical signs. Body weight and feed consumption measurements and a detailed examination for clinical signs were performed once a week, and an ophthalmoscopic examination was performed just prior to termination. Haematological and clinical chemistry parameters were measured at study termination. At necropsy, the weights of selected organs were recorded, assessed by gross examination and examined histopathologically.

There were no treatment-related effects on mortality, feed consumption, haematological or clinical chemistry parameters, ophthalmoscopic parameters, organ weights or gross pathological and histopathological parameters. There were no adverse effects on clinical signs, body weight or body weight gain. No neurotoxicity battery or cholesterol measurements were conducted in this study.

The no-observed-adverse-effect level (NOAEL) was 7000 ppm (equal to 1212.4 mg/kg bw per day), the highest dose tested (Pinto, 1997a).

Rats

In a 90-day study, groups of 12 Alpk:APfSD rats of each sex per dose received mesotrione (purity 93.3%) in the diet at a dose level of 0, 1, 125, 1250 or 12 500 ppm (equal to 0, 0.09, 10.96, 112.09 and 1110.86 mg/kg bw per day for males and 0, 0.10, 12.81, 125.58 and 1212.53 mg/kg bw per day for females, respectively). Animals were observed daily for morbidity, moribundity and overt clinical signs. Body weight and feed consumption measurements and a detailed examination for clinical signs were performed once per week. An ophthalmoscopic examination was performed prior to treatment and during the week prior to termination. Clinical chemistry, haematological and urine analysis parameters were measured at study termination. At necropsy, the weights of selected organs were recorded, assessed by gross examination and examined histopathologically.

There were no effects on mortality. Body weights were decreased by 8%, 7% and 16% and body weight gains were decreased by 12%, 11% and 23% compared with controls in males at 125, 1250 and 12 500 ppm, respectively. In females, body weight was decreased by 10% and body weight gain was decreased by 22% at 12 500 ppm. Feed efficiency was decreased in males at doses of 125 ppm and above.

Clinical signs of toxicity were limited to purple and/or yellow staining on the tray papers at doses of 1250 ppm and higher. In the ophthalmological examination, there was an increase in corneal opacity and vascularization in males (0/12, 0/12, 10/12, 10/12, 9/12) at doses of 125 ppm and higher and in females (0/12, 0/12, 0/12, 4/12, 9/12) at doses of 1250 ppm and higher.

The NOAEL was 1 ppm (equal to 0.09 mg/kg bw per day) in males and 125 ppm (equal to 12.81 mg/kg bw per day) in females. The lowest-observed-adverse-effect level (LOAEL) was 125 ppm in males (equal to 10.96 mg/kg bw per day), based on increased corneal opacity and

vascularization and decreased body weight and feed efficiency, and 1250 ppm in females (equal to 125.58 mg/kg bw per day), based on increased corneal opacity and vascularization (Horner, 1995).

In a 13-week study, groups of 12 Alpk:APfSD rats of each sex per dose received mesotrione (purity 96.8%) in the diet at a dose level of 0, 2.5, 5.0, 7.5 or 150 ppm (equal to 0, 0.21, 0.41, 0.63 and 12.46 mg/kg bw per day for males and 0, 0.23, 0.47, 0.71 and 14.48 mg/kg bw per day for females, respectively). Animals were observed daily for morbidity, moribundity and overt clinical signs. Body weight and feed consumption measurements and a detailed examination for clinical signs were performed once per week. An ophthalmoscopic examination was performed prior to treatment and during the week prior to termination. Clinical chemistry, haematological and urine analysis parameters were measured at study termination. At necropsy, the weights of selected organs were recorded, assessed by gross examination and examined histopathologically.

There were no effects on mortality or body weight. Clinical signs of toxicity consist of an increase in cloudy eyes in males at doses of 7.5 ppm and higher. There was one female with cloudy eyes at 150 ppm.

Under ophthalmoscopic examination, hazy corneal opacities, corneal opacities and vascularization were increased in males at doses of 7.5 ppm and higher, and corneal opacity was increased in females at 150 ppm.

The NOAEL was 5.0 ppm (equal to 0.41 mg/kg bw per day). The LOAEL was 7.5 ppm (equal to 0.63 mg/kg bw per day), based on increased cloudy eyes, corneal opacities and vascularization of the eyes in males (Pinto, 1997b).

Dogs

In a 13-week oral toxicity study, four Beagle dogs of each sex per dose were administered mesotrione (purity 96.8%) in gelatine capsules at a concentration of 0, 100, 600 or 1000 mg/kg bw per day. The animals were observed for clinical signs of toxicity, mortality and moribundity daily, feed consumption was measured daily and body weights were assessed weekly. Clinical chemistry and haematological parameters were measured prior to the initiation of dosing, at weeks 4 and 8 and prior to termination. Select organs were weighed at necropsy, assessed by gross observations and examined histopathologically.

There was no effect on mortality, and there were no adverse clinical signs of toxicity. Body weight gains were decreased compared with controls in males at 1000 mg/kg bw per day. Histopathologically, there was an increase in minimal/slight focal mesothelial proliferation of the atrium of the heart in two males at 1000 mg/kg bw per day.

The NOAEL was 600 mg/kg bw per day. The LOAEL was 1000 mg/kg bw per day, based on decreased body weights and increased mesothelial proliferation of the atrium of the heart in males (Brammer, 1997a).

In a short-term oral toxicity study, four Beagle dogs of each sex per dose were administered mesotrione (purity 97.6%) in capsules for 1 year at a concentration of 0, 10, 100 or 600 mg/kg bw per day. The animals were subjected to examinations 3 times per day for gross clinical signs, morbidity and moribundity, as well as weekly detailed clinical observations, weekly recording of body weight and daily recording of feed consumption. Ophthalmoscopic evaluations were performed at weeks 13, 26 and 39 and prior to termination. Blood samples were withdrawn for haematology and plasma clinical chemistry, and urine samples were collected from all animals predosing, at approximately weeks 4, 13 and 26 and prior to termination. Urine analysis was performed from samples collected prior to study initiation and during weeks 26 and 52. The animals were killed and subjected to necropsy and postmortem examination of major organs and tissues. Organs were weighed, and a full range of tissues was preserved, processed and examined by light microscopy. No measure of blood clotting potential was taken, and the spleen and uterus were not weighed.

Clinical signs of irritation and toxicity consisted of interdigital cysts at doses of 100 mg/kg bw per day and above in males and 600 mg/kg bw per day in females, dry sores or abrasions in the paws or limbs in all treated groups of males and yellow staining of the fur in all treated animals of both sexes. However, the findings were attributed to the presence of irritating metabolites of tyrosine in the urine (as confirmed by the presence of free phenolic acids in the urine of all treated dogs) and were not considered toxicologically relevant. Additionally, lime-green discoloration of the urine was noted in all treated animals, although the finding was not considered adverse.

Body weights were decreased in females at 600 mg/kg bw per day, and body weight gains were decreased in females at doses of 100 mg/kg bw per day and higher. There were no effects on body weight or body weight gain in males (Table 6).

Table 6. Mean body weight and body weight gain of dogs administered mesotrione for approximately 12 months

	Males (*n* = 6)				**Females (*n* = 6)**			
	0 mg/kg bw per day	**10 mg/kg bw per day**	**100 mg/kg bw per day**	**600 mg/kg bw per day**	**0 mg/kg bw per day**	**10 mg/kg bw per day**	**100 mg/kg bw per day**	**600 mg/kg bw per day**
Body weight (g)[a]								
Week 1	10.53 ± 0.76	10.18 ± 0.93	10.18 ± 0.89	10.38 ± 1.86	8.45 ± 1.05	8.35 ± 0.39	8.65 ± 0.73	8.60 ± 1.01
Week 53	13.13 ± 1.28	12.13 ± 1.68	12.40 ± 0.34	13.68 ± 1.62	10.80 ± 1.19	10.60 ± 0.54	10.40 ± 0.65	9.83 ± 1.10
Body weight relative to controls (%)	–	↓8	↓6	↑4	–	↓2	↓4	↓9
Overall body weight gain (g)	2.60	1.95	2.22	3.30	2.35	2.25	1.75	1.23
Overall body weight gain relative to controls (%)	–	↓25	↓15	↑27	–	↓4	↓26	↓48

bw: body weight; *: $P \leq 0.05$ (analysis of covariance)

[a] Mean ± standard deviation.

Source: Brammer (1997b)

There was a single incidence of lenticular opacity at the end of the treatment period in both high-dose males and females. There were also five observations in one animal in the control males; however, this was attributed to the remnants of a hyaline blood vessel, and the observation at the high dose was attributed to treatment (Table 7).

Plasma tyrosine levels were increased in all treated groups. In males, the increases were 2.6-fold, 8-fold and 10-fold in the low-, mid- and high-dose groups, respectively. In females, the increases were 2.5-fold, 13-fold and 16-fold in the low-, mid- and high-dose groups, respectively. The changes were considered adverse in the presence of treatment-related changes to the eye at 600 mg/kg bw per day.

At histopathological examination, there were single incidences of unilateral keratitis and periorbital haemorrhage in high-dose males and unilateral corneal erosion in high-dose females (Table 8).

The LOAEL was 600 mg/kg bw per day, based on keratitis and lenticular/corneal opacities and increased plasma tyrosine in males and females and decreased body weight in females only. The NOAEL was 100 mg/kg bw per day (Brammer, 1997b).

Table 7. Ophthalmoscopic observations in dogs administered mesotrione for approximately 12 months

Observation	Males				Females			
	0 mg/kg bw per day	10 mg/kg bw per day	100 mg/kg bw per day	600 mg/kg bw per day	0 mg/kg bw per day	10 mg/kg bw per day	100 mg/kg bw per day	600 mg/kg bw per day
Lenticular opacity								
No. of animals affected	1/4[a]	0/4	0/4	1/4	0/4	0/4	0/4	1/4
Total no. of observations	5	0	0	1	0	0	0	1
Range of first incidence (weeks)	Pre-52	–	–	52	–	–	–	47

bw: body weight

[a] Attributed to the remnant of hyaline blood vessel.

Source: Brammer (1997b)

Table 8. Incidence of selected histopathological lesions in dogs administered mesotrione for 1 year

Histopathological lesion	Males (n = 4)				Females (n = 4)			
	0 mg/kg bw per day	10 mg/kg bw per day	100 mg/kg bw per day	600 mg/kg bw per day	0 mg/kg bw per day	10 mg/kg bw per day	100 mg/kg bw per day	600 mg/kg bw per day
Eye								
Unilateral keratitis	0	0	0	1	0	0	0	0
Periorbital haemorrhage	0	0	0	1	0	0	0	0
Unilateral corneal erosion	0	0	0	0	0	0	0	1
Skin								
Interdigital dermatitis/folliculitis	0	1	2	2	0	0	0	2

bw: body weight

Source: Brammer (1997b)

(b) Dermal application

Rabbits

In a 21-day dermal toxicity study, groups of five New Zealand White rabbits of each sex per dose were treated dermally with mesotrione (purity 96.8%) applied to an approximately 15 cm × 13 cm area of the body surface at a dose of 0, 10, 500 or 1000 mg/kg bw per day for 3 weeks. The duration of treatment was 6 hours daily, with occlusion, 5 days/week. The animals were observed for clinical signs and skin irritation. Animals were weighed daily, and feed consumption was monitored daily and calculated as a weekly mean. An ophthalmoscopic examination was performed prior to study initiation and 2 days prior to study termination. Pretreatment and terminal blood samples were subjected to haematological and plasma chemistry analysis. Following termination, animals were subjected to necropsy and postmortem examination of major organs and tissues, including organ weights and histopathology of control and high-dose animals and any gross lesions.

There were no systemic signs of toxicity at the highest dose tested. Slight erythema was noted at 500 and 1000 mg/kg bw per day in males and females.

The NOAEL in males and females for systemic toxicity was 1000 mg/kg bw per day, the highest dose tested (Lees, 1997).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a chronic toxicity study in mice, mesotrione (purity 96.8%) was administered in the diet to 60 C57BL/10Jf CD-1 mice of each sex per dose at 0, 10, 50, 350 or 7000 ppm (equal to 0, 1.5, 7.8, 56.2 and 1114 mg/kg bw per day for males and 0, 2.1, 10.3, 72.4 and 1495 mg/kg bw per day for females, respectively) for up to 1 year. Twenty animals of each sex per dose were terminated after 3 and 6 months of treatment, with the 3-month results reported separately (see section 2.2(a); Pinto, 1997a). Detailed clinical signs were recorded weekly, and animals were observed daily for signs of overt toxicity and twice daily for morbidity and mortality. Body weights were recorded prior to treatment, weekly for the first 14 weeks of the study and monthly thereafter. Feed consumption was recorded weekly for 13 weeks of the study, in week 16 and then once every 4 weeks thereafter. Ophthalmological examinations were performed in males the week prior to termination in the control and high-dose animals in the 3- and 12-month study groups and in females the week prior to termination in all dose groups at 12 months. Haematological and clinical chemistry parameters were determined in all animals of each sex per dose at 14, 27 or 53 weeks via cardiac puncture at termination. Urine was collected over a period of 10–18 hours from each cage of surviving mice during weeks 13, 26 and 52. All animals were subjected to necropsy, postmortem examination and tissue preservation. Organ weights were recorded for 10 animals of each sex per dose from terminations at weeks 14 and 53. Lungs, liver, gallbladder and kidneys from both sexes and adrenal glands from males only from all groups from the 12-month scheduled termination were submitted for histology.

Body weight and body weight gain were decreased in males at 7000 ppm throughout treatment and in females at 7000 ppm in weeks 1–4. There were no effects on mortality, clinical signs of toxicity, ophthalmoscopy, haematology, clinical chemistry, urine analysis or postmortem findings. There was no evidence of oncogenicity.

The LOAEL for chronic toxicity was 7000 ppm (equal to 1114 mg/kg bw per day), based on decreased body weight and body weight gain in males. The NOAEL for chronic toxicity was 350 ppm (equal to 56.2 mg/kg bw per day) (Pinto, 1997c).

In an 18-month combined chronic toxicity and carcinogenicity study in mice, mesotrione (purity 96.8%) was administered in the diet to 55 C57BL/10Jf CD-1 Alpk mice of each sex per dose at 0, 10, 350 or 3500/7000 ppm (equal to 0, 1.4, 49.7 and 897.7 mg/kg bw per day for males and 0, 1.8, 63.5 and 1103 mg/kg bw per day for females, respectively) for 80 weeks. Animals received 3500 ppm mesotrione for the first 7 weeks of the study, but that dose was increased to 7000 ppm for the remainder of the study because of a lack of effects on body weight or feed consumption. Detailed clinical signs were recorded weekly, and animals were observed daily for signs of overt toxicity and twice daily for morbidity and mortality. Body weights were recorded prior to treatment, weekly for the first 12 weeks of the study and once every 2 weeks thereafter. Feed consumption was recorded for each cage of five mice weekly for 12 weeks of the study and then once every 4 weeks thereafter. Haematological parameters were determined in all animals of each sex per dose at 53 weeks from the tail vein and at study termination via cardiac puncture. All animals were subjected to necropsy, postmortem examination and tissue preservation. Organ weights were recorded for liver, kidneys, testes, brain and adrenal glands.

There was no evidence of carcinogenicity. There were no effects on mortality, clinical observations, haematology or postmortem findings. Body weight and body weight gain were decreased by 7% and 18%, respectively, in males given a dose of 3500 ppm. Feed efficiency was decreased in the same group by 16%. There were no adverse effects on body weight or feed efficiency in females.

The LOAEL for toxicity was 3500 ppm (equal to 897.7 mg/kg bw per day), based on decreased body weight, body weight gain and feed efficiency in males. The NOAEL was 350 ppm (equal to 49.7 mg/kg bw per day). The NOAEL for carcinogenicity was 3500/7000 ppm (equal to 897.7 mg/kg bw per day), the highest dose tested (Rattray, 1997).

Rats

In a combined chronic toxicity and carcinogenicity study in rats, mesotrione (purity 96.8%) was administered in the diet to 64 Alpk:APfSD (Wistar-derived) rats of each sex per dose at 0, 7.5, 100 or 2500 ppm (equal to 0, 0.48, 6.48 and 159.9 mg/kg bw per day for males and 0, 0.57, 7.68 and 189.5 mg/kg bw per day for females, respectively) for a period of up to 105 weeks. A satellite group of 12 preselected animals of each sex per dose was terminated at 52 weeks to assess chronic toxicity. In addition, 20 rats of each sex per group were assigned to dose levels of 1.0 or 2.5 ppm (equal to 0.06 and 0.16 mg/kg bw per day for males and 0.08 and 0.19 mg/kg bw per day for females, respectively) for a period of up to 105 weeks, to aid in the assessment of ocular toxicity only. The animals were observed twice daily for viability, clinical signs were recorded daily and a detailed physical examination was performed weekly. Body weights and feed consumption were recorded weekly for 15 weeks and biweekly thereafter. Feed consumption was measured for each cage of four rats on a weekly basis for 14 weeks, in week 16 and once every 4 weeks thereafter. An ophthalmological examination was conducted on all main study animals in all groups, including the 1.0 and 2.5 ppm groups, prior to study initiation, during study weeks 26, 52 and 78 and during the week prior to study termination. Blood samples taken from 13 predesignated animals of each sex dosed at 0, 7.5, 100 and 2500 ppm at weeks 14, 27, 53 and 79 were subjected to haematological and clinical chemistry analyses. Urine analysis was performed during study weeks 13, 26, 52, 78, 97 (males only) and 104 (females only) from the same predesignated animals as above. All animals dosed at 0, 7.5, 100 and 2500 ppm, including decedents, were subjected to necropsy, postmortem examination and tissue preservation. Select organs of all interim kill animals and terminal animals were weighed. Major organs and tissues from the control and high-dose groups and premature decedents from all groups, gross lesions from all animals and liver, lungs and kidneys from all animals were processed and examined by light microscopy. Blood clotting potential was not examined. The uterus and ovaries were weighed together, and only from animals killed at 24 months. The pharynx and larynx were not examined histopathologically. Eyes from the 1.0 and 2.5 ppm groups were preserved in Davidson's fixative and examined histopathologically.

There was no evidence of mortality, and there were no adverse changes in haematological, clinical chemistry or urine parameters. There were no adverse effects on organ weights in the interim or terminal kill animals.

Signs of clinical toxicity consisted of cloudy eyes at doses of 7.5 ppm and higher in males and of 100 ppm and higher in females, yellow and/or purple staining of tray papers at 2500 ppm in both sexes and increased incidence of dry sore in males and urine staining in both sexes at doses of 100 ppm and higher. Body weights and body weight gains were decreased in males at doses of 7.5 ppm and above; there were no adverse effects on body weight or body weight gain in females. Feed consumption was decreased in males and females at 2500 ppm.

In males, there was an increase in hazy corneal opacities, corneal opacities, vascularization and ghost vascularization at doses of 7.5 ppm and above. In females, vascularization and ghost vascularization were increased at doses of 100 ppm and above, and opacities were comparable with those in controls. In interim kill animals, there was an increase in opaque eyes at doses of 100 ppm and above in males and at 2500 ppm in females. In terminal animals, opaque and/or cloudy and/or vascularized corneal lesions were increased in males at doses of 7.5 ppm and above and in females at doses of 100 ppm and above. Additional findings noted for males at doses of 7.5 ppm and higher were enlarged adrenals, reduced seminal vesicles, pale livers, renal cysts and roughened renal surface. For females, there was an increased incidence of pale kidneys at 100 and 2500 ppm and an increased incidence of pale livers at 2500 ppm.

Histopathological examination revealed an increased incidence of keratitis in the 100 and 2500 ppm groups (both sexes) and in the 7.5 ppm group (males only); hepatocyte fatty vacuolation in the 100 and 2500 ppm groups (both sexes) and in the 7.5 ppm group (males only); thyroid follicular cysts for males in the 100 and 2500 ppm groups; follicular cysts with hyperplastic epithelium for males at all dose levels and for females in the 2500 ppm group; and thyroid squamous cysts for females in the 100 and 2500 ppm groups. At 2500 ppm, there was an increase in follicular cell adenomas in females. Follicular cell carcinomas were also increased, but were increased above the historical control range in all dose groups, including concurrent controls, and the increase was not considered related to treatment (Table 9).

Table 9. Incidence of selected histopathological lesions in rats administered mesotrione for 2 years

	Males (n = 4)				Females (n = 4)			
Histopathological lesion	**0 ppm**	**7.5 ppm**	**100 ppm**	**2 500 ppm**	**0 ppm**	**7.5 ppm**	**100 ppm**	**2 500 ppm**
Liver								
Hepatocyte fatty vacuolation	1/64	4/64	2/64	7/64	0/64	0/64	2/64	6/64
Thyroid								
Follicular cysts	1/64	0/63	3/64	5/64	0/64	0/64	1/62	0/64
Follicular cysts with hyperplastic epithelium	1/64	5/63	7/64	5/64	0/64	0/64	1/62	3/64
Thyroid squamous cysts	5/64	8/63	4/64	6/64	0/64	1/64	2/62	5/64
Follicular cell adenomas	0/64	1-63	3/64	1/64	0/64	1/64	1/64	4/64
Follicular cell carcinomas	0/64	1/63	0/64	1/64	5/64	4/64	2/64	6/64
Total thyroid tumours	2/64	2/63	3/64	2/64	5/64	5/64	3/64	10/64

ppm: parts per million

Source: Brammer (1997c)

The LOAEL was identified as 7.5 ppm (equal to 0.48 mg/kg bw per day), the lowest dose tested, based on an increased incidence of ocular effects noted at clinical, ophthalmological, gross or histopathological examinations, body weight changes and histopathological changes noted in the liver and thyroid in males. A NOAEL could not be determined There was no evidence of carcinogenicity (Brammer, 1997c).

2.4 Genotoxicity

A range of GLP-compliant studies of the genotoxicity of mesotrione was conducted to assess its potential for inducing chromosomal aberration, gene mutation and reverse gene mutation. There was no evidence for genotoxicity or mutagenicity in the available studies in the presence of metabolic activation (+S9) (summarized in Table 10); however, in the absence of metabolic activation (−S9), the in vitro mammalian chromosomal aberration test was equivocal. Overall, mesotrione did not demonstrate any genotoxic potential.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Mice

In a two-generation reproductive toxicity study in mice, mesotrione (purity 96.8%) was administered in the diet to 26 CD-1 mice of each sex per dose at a concentration of 0, 10, 50, 350, 1500 or 7000 ppm (equal to 0, 2.1, 10.2, 71.4, 311.8 and 1472 mg/kg bw per day for males and 0, 2.1, 10.0, 71.3, 301.6 and 1439 mg/kg bw per day for females; Table 11). After 8 weeks, the animals were mated and allowed to rear the ensuing F_{1A} litters to weaning. The breeding programme was repeated

Table 10. Genotoxicity studies with mesotrione

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* and *Escherichia coli*	0, 100, 200, 500, 1 000, 2 500 or 5 000 µg/plate ±S9	98.1	Negative	Callander (1993)
Mammalian cell cytogenetics	Human lymphocytes	0, 250, 1 000 or 2 000 µg/mL +S9	98.1	Negative	Griffiths (1994a)
		0, 250, 1 000 or 1 500/2 000 µg/mL –S9		Equivocal	
Mammalian cell gene mutation	L5178Y mouse lymphoma cells, *TK* locus	125, 250, 500 or 1 000 µg/mL	98.1	Negative	Clay (1994)
In vivo					
Mouse micronucleus	CD-1 mouse bone marrow, males and females	500 mg/kg bw Harvest time: 24 and 48 h	98.1	Negative	Griffiths (1994b)

bw: body weight; S9: 9000 × *g* supernatant fraction from liver homogenate from Aroclor-treated rats; TK: thymidine kinase

Table 11. Mean intake of mesotrione in the two-generation reproductive toxicity study in mice

	Dose (mg/kg bw per day)									
	F_0 generation					F_1 generation				
	10 ppm	50 ppm	350 ppm	1 500 ppm	7 000 ppm	10 ppm	50 ppm	350 ppm	1 500 ppm	7 000 ppm
Males premating	2.1	10.2	71.4	311.8	1 472	2.1	10.0	71.3	301.6	1 439
Females premating	2.4	12.0	84.4	371.6	1 632	2.4	11.4	80.5	353.8	1 673
Females gestation period	2.1	10.0	72.9	300.1	1 430	2.0	9.8	73.5	302.9	1 491
Females lactation period	13.4	70.1	481.6	2 001	8 726	13.2	65.8	422.4	1 879	8 260

bw: body weight; F_0: parental generation; F_1: first filial generation; ppm: parts per million

Source: Moxon (1997)

with the F_1 adults selected from the F_{1A} offspring to produce the F_{2A} litters after a premating period of at least 8 weeks. Selected F_{2A} pups were retained post-weaning (F_2 adults) for the measurement of preputial separation only. Diets containing mesotrione were fed continuously throughout the study. The following investigations were undertaken in the adults: clinical observations, body weights (including during pregnancy and lactation for females), feed consumption (including during pregnancy and lactation for females) and utilization, reproductive performance, preputial separation (F_1 and F_2 adults only), postmortem examination, organ weights, histopathology and plasma tyrosine levels (some F_1 adults). The following investigations were undertaken in the litters/pups: numbers of pups at birth and up to/including day 29, pup survival, pup and litter weights, clinical condition, postmortem examination of selected pups, organ weights and plasma tyrosine levels (some F_{2A} pups) for 8 weeks prior to mating and through lactation and weaning of the F_1 offspring. Groups of 26 male and 26 female F_1 generation offspring were then similarly treated through 21 days of lactation of the F_2 offspring.

There were no effects on mortality. Clinical signs of toxicity were limited to a single incidence of cloudy eye in F_0 high-dose males and multiple incidences in F_1 high-dose males and females (Table 12). This was corroborated by findings in the gross necropsy and increases in unilateral cataractous changes in 7000 ppm males in the F_0 generation and unilateral/bilateral cataractous changes in 7000 ppm males and females in the F_1 generation. There were no effects on body weight or body weight gain in adult animals. Body weights were lower in high-dose animals in the F_1 generation; however, this is considered to be a result of lower pup body weights. There were no treatment-related or adverse effects on feed consumption or feed efficiency.

Table 12. Summary of eye-related changes in parental mice from the two-generation reproductive toxicity study

	Incidence of finding					
Finding	**0 ppm**	**10 ppm**	**50 ppm**	**350 ppm**	**1 500 ppm**	**7 000 ppm**
Males F_0						
Clinical signs of toxicity						
Cloudy eyes	0/26	0/26	0/26	0/26	0/26	1/26
Gross pathology						
Opaque/cloudy eyes	0/26	0/26	0/26	1/26	0/26	3/26
Histopathological examination						
Unilateral cataractous change	0/26	0/22	0/25	0/26	0/26	3/26
Males F_1						
Clinical signs of toxicity						
Cloudy eyes	0/26	1/26	0/26	1/26	0/26	4/26
Gross pathology						
Opaque/cloudy eyes	0/26	2/26	0/26	2/26	1/26	5/26
Histopathological examination						
Unilateral cataractous change	0/26	0/26	0/25	0/24	0/26	7/25
Bilateral cataractous change	0/26	0/26	0/25	0/24	0/26	1/25
Females F_1						
Clinical signs of toxicity						
Cloudy eyes	0/26	0/26	0/26	0/26	1/26	6/26
Gross pathology						
Opaque/cloudy eyes	1/26	0/26	0/26	0/26	1/26	5/26
Histopathological examination						
Unilateral cataractous change	0/26	0/26	0/26	0/26	0/26	4/26
Bilateral cataractous change	0/26	0/26	0/26	0/26	0/26	2/26

F_0: parental generation; F_1: first filial generation; ppm: parts per million
Source: Moxon (1997)

Estrous cycle parameters and sperm parameters were not measured. There was a decrease in successful matings (i.e. the number of females producing at least one live pup) in F_0 females at and above 350 ppm (Table 13). The incidence of successful matings in all groups, including controls, was lower than expected in the F_1 animals, and therefore the change in the F_0 animals was of unknown significance.

Table 13. Successful matings in mice treated with mesotrione

	Incidence of successful matings					
	0 ppm	**10 ppm**	**50 ppm**	**350 ppm**	**1 500 ppm**	**7 000 ppm**
F_0 females						
Dams with at least one live pup	24/26	25/26	23/24	20/26	22/26	20/26
F_1 females						
Dams with at least one live pup	21/26	20/26	21/25	20/24	21/26	19/26

F_0: parental generation; F_1: first filial generation; ppm: parts per million

Source: Moxon (1997)

Clinical signs of toxicity in the offspring were limited to an increased incidence of opaque eyes observed in one F_{2A} litter at 7000 ppm (Table 14). There were no effects on viability. Mean pup body weight was lower in the 1500 and 7000 ppm groups (both generations and both sexes) on lactation days 22 and 29 and slightly lower for F_{2A} pups in the 7000 ppm group on lactation day 8. F_{1A} males in the 10 ppm dose group had decreased body weights, but the finding was not consistent across sexes or generations; therefore, the change was not considered treatment related. In the F_{1A} generation, there was an increase in unilateral and bilateral cataractous changes at 7000 ppm in both sexes. In the F_{2A} generation, changes at necropsy consisted of an increase in opaque/cloudy eyes in the 1500 and 7000 ppm groups of both sexes and an increased incidence of unilateral/bilateral cataractous change in the 7000 ppm group in both sexes and in the 1500 ppm group in males only. Plasma tyrosine levels were increased by factors of 3.7 in males and 2.6 in females at 10 ppm. At 7000 ppm, plasma tyrosine levels were increased by factors of 6.9 in males and 6.4 in females.

The LOAEL for parental toxicity in CD-1 mice was 7000 ppm (equal to 1439 mg/kg bw per day), based on clinical, gross and histopathological changes to the eyes (opaque/cloudy eyes; cataractous change) and increased plasma tyrosine levels. The NOAEL for parental toxicity was 1500 ppm (equal to 301.6 mg/kg bw per day).

The NOAEL for reproductive toxicity in CD-1 mice was 7000 ppm (equal to 1439 mg/kg bw per day), the highest dose tested.

The LOAEL for offspring toxicity in CD-1 mice was 1500 ppm (equal to 301.6 mg/kg bw per day), based on decreased body weight and body weight gain, clinical, gross and histopathological changes to the eyes (opaque/cloudy eyes; cataractous change) and increased plasma tyrosine levels. The NOAEL was 350 ppm (equal to 71.3 mg/kg bw per day) (Moxon, 1997).

Rats

In a three-generation reproductive toxicity study in rats, mesotrione (purity 96.8%) was administered in the diet to 26 Alpk:APfSD rats of each sex per group at a concentration of 0, 2.5, 10, 100 or 2500 ppm (equal to 0, 0.3, 1.1, 11.6 and 278.1 mg/kg bw per day for males and 0, 0.3, 1.1, 11.7 and 297.2 mg/kg bw per day for females, respectively). After 10 weeks, the animals were mated and allowed to rear the ensuing F_{1A} litters to weaning. The breeding programme was repeated with the

Table 14. Summary of eye-related changes in offspring from the two-generation reproductive toxicity study in mice

	Incidence of finding					
Finding	**0 ppm**	**10 ppm**	**50 ppm**	**350 ppm**	**1 500 ppm**	**7 000 ppm**
F_{1A} litters						
Clinical signs of toxicity						
No. of pups (no. of litters)	*282 (25)*	*321 (25)*	*300 (23)*	*252 (20)*	*279 (21)*	*236 (20)*
Discharge from eyes	0 (0)	4 (3)	1 (1)	4 (3)	5 (3)	11 (5)
Histopathological examination (no. affected/no. examined)						
Unilateral cataractous change						
- Males	0/37	0/24	0/11	0/12	0/14	2/30
- Females	0/40	0/21	0/17	0/13	0/13	2/30
Bilateral cataractous change						
- Males	0/37	0/24	0/11	0/12	0/14	2/30
- Females	0/40	0/21	0/17	0/13	0/13	0/30
F_{2A} litters						
Clinical signs of toxicity						
No. of pups (no. of litters)	*249 (21)*	*248 (20)*	*249 (21)*	*222 (20)*	*242 (21)*	*228 (19)*
Discharge from eyes	0 (0)	3 (3)	0 (0)	3 (3)	2 (2)	11 (5)
Opaque eyes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (1)
Macroscopic examination (no. affected/no. examined)						
Opaque/cloudy eyes						
- Males	0/30	1/28	0/31	0/30	4/30	10/33
- Females	1/31	0/29	0/31	0/30	3/30	3/31
Histopathological examination (no. affected/no. examined)						
Unilateral cataractous change						
- Males	0/30	0/16	0/11	0/11	2/18	3/33
- Females	0/32	0/18	0/13	0/12	0/18	2/31
Bilateral cataractous change						
- Males	0/30	0/16	0/11	0/11	0/18	8/33
- Females	0/32	0/18	0/13	0/12	0/18	1/31

F_0: parental generation; F_1: first filial generation; ppm: parts per million

Source: Moxon (1997)

F_1 adults selected from the F_{1A} offspring to produce the F_{2A} litters after a 10-week premating period. A further generation (F_2) was then selected from the F_{2A} litters in order to clarify findings seen in the first two generations. In the F_2 generation, all animals were fed experimental diet until week 14, when approximately half of the animals in all groups continued with the same treatment while the remainder of the animals were assigned to a recovery subgroup and fed control diet. At week 18, the subgroups were mated to produce the F_{3A} litters. Ophthalmoscopic examinations were included in all generations. Test diets were fed continuously throughout the study with the exception of the recovery animals in the third generation referred to above (F_2 adults and F_{3A} offspring). The following investigations were undertaken in the adults: clinical observations, body weights (including during pregnancy and lactation for females), feed consumption (including during pregnancy and lactation for females) and utilization, reproductive performance, preputial separation (F_1 and F_2 adults only), postmortem examination and organ weights. Estrous cycle length and periodicity and sperm parameters were not measured. The following investigations were undertaken in the litters/pups: numbers of pups at birth and up to/including day 29, pup survival, pup and litter weights, clinical condition, postmortem examination of selected pups, organ weights (except F_{3A} pups) and ophthalmoscopy (F_{3A} pups only).

In the parental animals, there were no effects on body weight gain, feed intake, feed efficiency or organ weights. Eye lesions were observed in the clinical observations, ophthalmoscopic examination and gross and histopathological examinations at 10 ppm in males and in both sexes at and above 100 ppm. Changes to the eyes consisted of cloudy/opaque eyes, corneal opacity and/or keratitis with corneal vascularization. Recovery animals were noted to have ghost vascularization indicating healed lesions in both sexes at 2500 ppm and in males at 100 ppm. At gross necropsy, there was an increase in bilateral hydronephrosis in the F_1 generation at 2500 ppm in males and females and in the F_2 adults at 2500 ppm in males only following continuous treatment and in both sexes in the recovery group. Plasma tyrosine levels were increased to a toxicologically significant extent at doses of 10 ppm and above in F_1 animals. In the recovery F_2 adults, plasma tyrosine levels were unchanged from control values.

Litter size and pup survival to day 22 were decreased at 2500 ppm in the F_{1A}, F_{2A} and F_{3A} continuous treatment litters. In addition, whole litter loss was increased and the percentage of pups born live was decreased in the F_{2A} and F_{3A} continuous treatment litters. There were no other treatment-related effects on reproductive function or reproductive performance.

Effects on offspring consisted of increased incidence of cloudy/opaque eyes, keratitis and/or corneal vascularization in all treated groups of males and at doses of 100 ppm and above in females in litters exposed to mesotrione in utero. Cataractous change was noted in both sexes at 2500 ppm in the F_{1A}, F_{2A} and F_{3A} continuous treatment litters. Grossly, there were increased incidences of bilateral renal pelvic dilatation in both sexes at 2500 ppm in continuously treated F_{3A} litters. Histopathologically, there was an increase in bilateral hydronephrosis at 2500 ppm in F_{1A} and F_{2A} litters and at 2500 ppm in F_{3A} continuous treatment litters. There were no effects of treatment in the absence of mesotrione exposure in utero. Plasma tyrosine levels were increased in F_{3A} pups under continuous treatment at all dose levels tested. Plasma tyrosine levels in F_{3A} pups in the recovery groups were similar to the control values at all dose levels.

The LOAEL for parental toxicity was 10 ppm (equal to 1.1 mg/kg bw per day), based on clinical, ophthalmological, gross and pathological changes in the eyes and increased plasma tyrosine levels in males and females. The NOAEL was 2.5 ppm (equal to 0.3 mg/kg bw per day).

The LOAEL for reproductive toxicity was 2500 ppm (equal to 297.2 mg/kg bw per day), based on decreased litter size, decreased pup survival to day 22, decreased percentage of pups born live and an increase in whole litter loss. The NOAEL was 100 ppm (equal to 11.7 mg/kg bw per day).

The LOAEL for offspring toxicity was 2.5 ppm (equal to 0.3 mg/kg bw per day), the lowest dose tested, based on clinical, ophthalmological, gross and histopathological changes to the eyes and increased plasma tyrosine levels. A NOAEL for offspring toxicity was not identified (Milburn, 1997b).

(b) Developmental toxicity

Mice

In a developmental toxicity study, mesotrione (purity 96.8%) was administered to mated Alpk:APfCD-1 female mice via gavage from days 5 to 18 of gestation at a dose level of 0 (two control groups), 10, 60, 150 or 600 mg/kg bw per day at a dosing volume of 10 mL/100 g bw in water. The animals were killed on day 19 after mating for reproductive assessment and external fetal examination. Clinical signs and body weight were recorded. Adult females were examined macroscopically at necropsy on day 29 after mating, and all fetuses were examined macroscopically at maternal necropsy.

There were no clinical signs of toxicity, no effects on body weight or feed consumption and no treatment-related changes noted during the gross necropsy. There were no changes to the caesarean section parameters.

There was a slight increase in fetuses with minor external anomalies in the 600 mg/kg bw per day dose group; however, there was no increase in minor anomalies as a function of litters (Table 15). There were no other treatment-related changes to fetal parameters.

Table 15. External/visceral examinations in a developmental toxicity study in mice

	Litter (fetal) incidence					
	0 mg/kg bw per day	**0 mg/kg bw per day**	**10 mg/kg bw per day**	**60 mg/kg bw per day**	**150 mg/kg bw per day**	**600 mg/kg bw per day**
No. of litters (fetuses) examined	*24 (299)*	*26 (315)*	*24 (302)*	*25 (315)*	*25 (313)*	*23 (294)*
Total malformations	3 (3)	1 (6)	2 (2)	2 (2)	2 (2)	3 (4)
Total minor anomalies	11 (12)	9 (17)	10 (16)	11 (17)	12 (21)	12 (26*)
Total variants	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

bw: body weight, *: $P \leq 0.05$ (Student's *t*-test)

Source: Moxon (1999a)

A LOAEL for maternal toxicity could not be identified. The NOAEL for maternal toxicity was 600 mg/kg bw per day, the highest dose tested.

A LOAEL for embryo and fetal toxicity could not be identified. The NOAEL for embryo and fetal toxicity was 600 mg/kg bw per day, the highest dose tested (Moxon, 1999a).

Rats

In a developmental toxicity study, mesotrione (purity 96.8%) was administered to mated female Alpk:APfSD (Wistar-derived) rats (25 per dose) via gavage from day 7 to day 16 of gestation at a dose level of 0, 100, 300 or 1000 mg/kg bw per day at a dosing volume of 1.0 mL/100 g bw in deionized water. The animals were terminated on day 22 after mating for reproductive assessment and fetal examination. Clinical signs, body weight and feed consumption were recorded. Adult females were examined macroscopically at necropsy on day 20 after mating, and all fetuses were examined macroscopically at maternal necropsy and subsequently by detailed internal visceral or skeletal examination. Maternal body weight and feed consumption were not analysed statistically, and the homogeneity and stability of the test substance in the vehicle were not determined.

There were no maternal deaths. There was a dose-related decrease in body weight gain in all treated animals during the dosing period and throughout the study period in 1000 mg/kg bw per day dams (Table 16). Feed consumption was decreased during dosing and decreased in the first 3 days of the post-dosing period in all treated animals and was comparable with control values thereafter.

Table 16. Maternal body weight and body weight gain in pregnant rats (n = 24 per dose) given mesotrione

	Body weight/body weight gain (g)			
	0 mg/kg bw per day	**100 mg/kg bw per day**	**300 mg/kg bw per day**	**1 000 mg/kg bw per day**
Initial body weight	268.3 ± 16.9	271.0 ± 16.2	269.6 ± 17.2	261.6 ± 17.8
Pretreatment gain				
Days 1–7	28	26.6	27.5	28.0
Treatment gain				
Days 7–10	16.0	13.3 (↓17)	9.6 (↓40)	8.1 (↓49)
Days 10–13	17.8	14.3 (↓20)	11.9 (↓33)	11.1 (↓38)
Days 13–16	22.7	21.2 (↓7)	21.2 (↓7)	19.4 (↓15)
Total gain, days 7–16	56.5	48.8 (↓14)	42.7 (↓24)	38.6 (↓32)
Post-treatment gain				
Days 16–20	40.1	40.8	38.7	40.1
Days 20–22	31.8	36.8	39.5	32.9
Final body weight	424.7 ± 22.6	424.0 ± 29.2	418.0 ± 30.0	401.3 ± 31.8* (↓6)
Gravid uterine weight	96.3 ± 14.5	94.2 ± 20.5	91.6 ± 19.2	90.0 ± 20.6
Corrected final body weight	328.4 ± 21.5	329.8 ± 27.1	326.4 ± 24.6	310.4 ± 26.5* (↓5)
Body weight gain				
Days 1 to 22	156.4	153.0	148.4 (↓5)	139.7 (↓11)
Days 7–22	128.4	126.4	120.9 (↓6)	111.7 (↓13)
Corrected body weight gain				
Days 1 to 22	60.1	58.8	56.8 (↓5)	49.7 (↓17)
Days 7–22	32.1	32.2	29.3 (↓9)	20.8 (↓35)

bw: body weight; *: $P < 0.05$ (analysis of covariance)

Source: Moxon (1999c)

Fetal weights were decreased in the 1000 mg/kg bw per day dose group; however, there were no other treatment-related effects on the caesarean section parameters.

Although there were no effects on external and skeletal malformations and all litters showed at least one minor skeletal anomaly and/or variant in control and treated dams, there was a dose-related and statistically significant increase in the number of fetuses exhibiting minor skeletal anomalies in all treated groups. These anomalies were generally related to a lack of ossification or supernumerary ribs and were determined to be a result of decreased body weight gain in the dams.

The LOAEL for maternal toxicity was 100 mg/kg bw per day, based on decreased body weight gain and feed consumption at the lowest dose tested. A NOAEL for maternal toxicity could not be identified.

The LOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, based on decreased ossification at the lowest dose tested. A NOAEL for embryo and fetal toxicity could not be identified (Moxon, 1999b).

Rabbits

In a developmental toxicity study in rabbits, mesotrione (purity 96.8%) was administered to time-mated female New Zealand White rabbits (20 per dose) via gavage, from day 8 to day 20 of gestation, at a dose level of 0, 100, 250 or 500 mg/kg bw per day at a dosing volume of 10 mL/kg bw in deionized water. The animals were killed on day 30 after mating for reproductive assessment and fetal examination. Clinical signs, body weight and feed consumption were recorded. Adult females were examined macroscopically at necropsy on day 30 after mating, and all fetuses were examined macroscopically at maternal necropsy and subsequently by detailed examination for external and visceral variations and abnormalities and skeletal variations and abnormalities, including ossification of the manus and pes.

Clinical signs of toxicity consisted of an increased incidence of red/brown urine at all dose levels and decreased defecation in the 250 and 500 mg/kg bw per day dose groups. Body weight gain was decreased by 34% at 500 mg/kg bw per day during the dosing period, although there were no changes in corrected final body weight or body weight gain.

In the caesarean section parameters, there was a slight increase in abortions in the 250 and 500 mg/kg bw per day dose groups (0/20, 1/20, 2/20, 2/20). Other than a large, but non-treatment-related, increase in preimplantation loss at 500 mg/kg bw per day, all other caesarean section parameters were comparable with control values.

Fetal examination revealed no increase in external or visceral malformations, anomalies or variants. There was no increase in skeletal malformations, and there was a decrease in minor skeletal anomalies. Although all litters, including controls, contained fetuses with delays in ossification, the number of fetuses with delayed ossification increased in all treated litters, with statistically significant increases at doses of 250 mg/kg bw per day and higher. These delays were also seen in the manus and pes assessment. Mean scores were comparable among all groups, but examination of individual scores indicated a dose-related reduction in the degree of ossification in all treated groups. Although the changes were considered treatment related, they were also considered transient and reversible and not considered to be adverse.

The LOAEL for maternal toxicity was 250 mg/kg bw per day, based on increased abortions and decreased defecation. The NOAEL for maternal toxicity was 100 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 500 mg/kg bw per day, the highest dose tested (Moxon, 1999c).

2.6 Special studies

(a) Neurotoxicity

In an acute neurotoxicity study, mesotrione (purity 97.6%) was administered to young adult Alpk:APfSD rats (10 of each sex per dose) via gavage at a dosing volume of 10 mL/kg bw in deionized water at a dose level of 0, 20, 200 or 2000 mg/kg bw and then maintained for a 15-day observation period. Feed consumption and body weights were recorded, and a functional observational battery of tests, including a quantitative assessment of motor activity, was performed on all animals predosing, 2 hours post-dosing and on days 8 and 15. Five animals of each sex in the control and high-dose groups were subjected to necropsy, postmortem examination, brain weight and dimensions recording, perfusion fixation and preservation of brain, dorsal root fibres and ganglia, ventral root fibres and spinal cord.

No premature deaths occurred. There were no clinical signs of toxicity and no effects on body weight or feed consumption. There were no treatment-related findings observed at the functional

observational battery or motor activity testing conducted at 2 hours, 8 days and 15 days post-dosing. Gross examination of the brain and histopathological examination of the central nervous system and the peripheral nervous system did not reveal any treatment-related findings.

The NOAEL for systemic toxicity and acute neurotoxicity was 2000 mg/kg bw, the highest dose tested (Horner, 1997a).

In a 13-week neurotoxicity study, mesotrione (purity 97.6%) was administered in the diet to groups of 10 male and 10 female Sprague-Dawley rats at a concentration of 0, 2.5, 100 or 5000 ppm (equal to 0, 0.20, 8.25 and 402.8 mg/kg bw per day for males and 0, 0.23, 9.29 and 466.6 mg/kg bw per day for females, respectively) for 3 months. Feed consumption and body weights were recorded weekly, and a functional observational battery of tests, including a quantitative assessment of motor activity, was performed on all animals predosing and during weeks 5, 9 and 14 post-dosing. Five animals of each sex in the control and high-dose groups were subjected to necropsy, postmortem examination, brain weight and dimensions recording, perfusion fixation and preservation of brain, dorsal root fibres and ganglia, ventral root fibres, spinal cord, skeletal muscle and gross lesions. In addition, an ophthalmological examination was performed during week 13.

There were no treatment-related mortalities. Clinical signs were limited to an increase in ocular opacity at doses of 100 ppm and above in males and at 5000 ppm in females. There was a slight decrease in final body weights at doses of 100 ppm and higher in males and females; however, the extent of the impact was not considered adverse. There were no effects on feed consumption, functional observational battery evaluations or motor activity measurements. There were no neurological effects noted in the postmortem examinations, and postmortem effects were limited to corneal opacity at 100 ppm and above in males and at 5000 ppm in females. In the ophthalmological examination, there were increases in corneal opacities, hazy corneal opacities and vascularization at doses of 100 ppm and higher in males and at 5000 ppm in females. Ghost vascularization was noted in females at doses of 100 ppm and above.

The LOAEL for systemic toxicity was 100 ppm (equal to 8.25 mg/kg bw per day), based on corneal opacity, hazy corneal opacity and vascularization in males and ghost vascularization in females. The NOAEL for systemic toxicity was 2.5 ppm (equal to 0.20 mg/kg bw per day). The NOAEL for neurotoxicity was 5000 ppm (equal to 402.8 mg/kg bw per day), the highest dose tested (Horner, 1997b).

(b) Mode of action studies

A number of studies have been conducted with mesotrione to investigate the ocular and systemic toxicity in rats and the differences in the systemic toxicity between rats and mice and to facilitate an understanding of the mode of action (MOA) of mesotrione in mammals. Appendix 1 contains further summaries of these mode of action studies within the human relevance framework developed by the International Life Sciences Institute (ILSI)/Health and Environmental Sciences Institute (HESI) workgroup.

In a 28-day dietary toxicity study, mesotrione (purity 100%) was administered in the diet to five Alpk:AP rats and five AP Swiss albino mice of unspecified sex per dose at 0, 1000, 7000 or 16 000 ppm (equivalent to 0, 100, 700 and 1600 mg/kg bw per day, respectively, for males and females) to rats and at 0, 1000, 3000 or 7000 ppm (equivalent to 0, 150, 450 and 1050 mg/kg bw per day, respectively, for males and females) to mice for 28 days. Clinical observations, body weights and feed consumption were recorded throughout the study. Blood samples were taken at termination and analysed for various clinical chemistry parameters. Livers were removed at termination and weighed, and portions were processed for liver biochemistry, routine histopathology and electron microscopy.

As a positive control, groups of three Alpk:AP rats and four AP Swiss albino mice were administered β-naphthoflavone, phenobarbitone, dexamethasone or methylclofenapate in standard inducer studies in which control animals received corn oil.

Mesotrione caused minimal cytochrome P450 induction at high doses and slight centrilobular hypertrophy in rats at the high dose of 16 000 ppm and slight liver hypertrophy at doses of 3000 ppm and above in mice. There were increases in proliferation of the smooth endoplasmic reticulum, but no evidence of peroxisome proliferation in either mice or rats. Ethoxycoumarin *O*-deethylation, pentoxyresorufin *O*-dependentylation and methoxyresorufin *O*-demethylation were increased in rats, whereas benzoxyresorufin *O*-debenzylation was increased in mice. However, as there was no evidence of hepatotoxicity, hepatomegaly or peroxisome proliferation, mesotrione was considered to be unlikely to cause liver tumours in rats or mice (Odum, 1997).

In a non-guideline dietary study to investigate the effects of L-tyrosine on the eye, eight male Alpk:APfSD weanling rats were fed a low-protein diet supplemented with 0%, 0.5%, 1%, 2.5% or 5% L-tyrosine for up to 21 days. Ophthalmoscopy was undertaken on days 2, 3, 4, 5, 6, 7, 8, 11, 12, 14, 18 and 21. Rats were killed when the majority of animals in each group showed corneal lesions. All remaining rats were killed on day 21. All rats were necropsied, and the eyes and Harderian glands were examined histopathologically.

There was no evidence of corneal lesions up to 21 days in animals given 1% L-tyrosine or less. In the 2.5% dose group, 2/8 animals showed evidence of corneal lesions by day 4, and 6/8 animals exhibited lesions by day 6. In the 5% dose group, 5/8 animals exhibited lesions on day 3, and 6/8 animals exhibited lesions on day 4. At histopathology, there was a slight increase in retinal rosettes and increased porphyrin at doses of 1% L-tyrosine and above; however, the changes were considered unrelated to tyrosine administration. In the animals dosed at 2.5% L-tyrosine and above, there were increases in minimal and slight keratitis and polymorphic filtration angle. At 5% L-tyrosine, there was an increase in minimal epithelial disorganization.

In conclusion, an increase in L-tyrosine was considered to induce the same corneal changes in Alpk:APfSD rats in the performing laboratory as seen in the literature regarding changes induced by triketones (Robinson, 1995).

In a non-guideline dietary study, eight female Alpk:APfSD (Wistar-derived) rats were fed diet containing 0 or 100 ppm mesotrione (purity 96.8%) with 0%, 0.5%, 1.0% or 2.5% tyrosine for 28 consecutive days (Table 17). Clinical observations, body weights and feed consumption were monitored throughout the study. Urine samples were taken for assessment of ketones after 24 hours, after 1 week and prior to scheduled termination. Blood samples were taken for assessment of plasma tyrosine after 24 hours, after 1 week and at scheduled termination. At the end of the scheduled period, the animals were killed and subjected to a postmortem examination. Liver and kidneys were weighed, and samples of kidney and liver were fixed and stored. Liver samples taken at termination were assayed for the activities of the enzymes tyrosine aminotransferase (TAT) and HPPD.

There was a statistically significant decrease in body weight and body weight gain in animals given 100 ppm mesotrione in conjunction with 2.5% tyrosine. There was a 6% decrease in body weight in animals given 100 ppm mesotrione in conjunction with 1.0% tyrosine, but the change was not statistically significant. Feed consumption was decreased in animals given 100 ppm mesotrione and 2.5% tyrosine. There were decreases in both parameters at other doses and time points, but there was no dose–response relationship, and the changes were considered adverse only in animals given both mesotrione and 2.5% tyrosine.

Corneal lesions were seen in all groups given mesotrione. There was a treatment-related increase in the severity of lesions with the addition of increasing amounts of tyrosine to the point where all animals given 100 ppm mesotrione and 2.5% tyrosine exhibited marked to moderate corneal

Table 17. Dose groups for the 28-day non-guideline study in female Alpk:APfSD (Wistar-derived) rats

Dose group	Mesotrione (ppm)	Tyrosine (%)
1	0	0
2	100	0
3	100	0.5
4	100	1.0
5	100	2.5
6	0	0.5
7	0	1.0
8	0	2.5

ppm: parts per million

Source: Milburn (1997a)

opacity and vascularization. In the absence of mesotrione, there were no changes to the eyes in any of the groups treated with tyrosine.

Plasma tyrosine levels were increased in all treated animals. In the presence of 100 ppm mesotrione, plasma tyrosine levels were at least an order of magnitude higher than those in animals given equivalent amounts of tyrosine in the absence of mesotrione (Table 18).

Table 18. Plasma tyrosine levels in female rats given mesotrione and/or tyrosine in diet

	Plasma tyrosine level (nmol/mL)							
Study period	**Control diet**	**100 ppm M**	**100 ppm M and 0.5% T**	**100 ppm M and 1.0% T**	**100 ppm M and 2.5% T**	**Control diet and 0.5% T**	**Control diet and 1.0% T**	**Control diet and 2.5% T**
24 hours	112.5 ± 12.53	1 503 ± 122.8	2 129 ± 98.99	2 579 ± 276.8	3 517 ± 872.3	136.4 ± 15.61	142.5 ± 12.85	268.9 ± 38.27
1 week	141.5 ± 5.019	1 243 ± 246.6	1 733 ± 323.7	2 291 ± 355.0	3 729 ± 756.1	160.5 ± 29.59	183.7 ± 29.56	244.6 ± 52.64
Termination	106.9 ± 8.790	1 189 ± 107.8	1 519 ± 65.69	2 803 ± 388.8	2 576 ± 866.2	129.5 ± 11.86	139.3 ± 27.58	187.6 ± 37.70

M: mesotrione; ppm: parts per million; T: tyrosine

Source: Milburn (1997a)

TAT activity was increased in all groups given mesotrione and in the group given 2.5% tyrosine in the absence of mesotrione. HPPD activity was decreased in all groups given mesotrione and not increased in groups given tyrosine in the absence of mesotrione.

At necropsy, relative liver weights were increased in all groups given mesotrione and not increased in groups given tyrosine in the absence of mesotrione. Relative kidney weights were increased only in the presence of mesotrione and 2.5% tyrosine. All animals given mesotrione exhibited cloudy eyes. There were no other treatment-related findings.

In conclusion, the administration of 100 ppm mesotrione in combination with tyrosine in the diet to female rats resulted in marked tyrosinaemia and associated ocular effects and changes in liver enzyme activities. There was some evidence of exacerbation of these effects with increasing tyrosine

levels. Generally, the effects seen were more marked than those observed when either 100 ppm mesotrione or tyrosine was given alone (Milburn, 1997a).

In a non-guideline dietary study to investigate dose–response patterns, 16 young adult male Alpk:APfSD (Wistar-derived) rats were fed a diet containing mesotrione (purity 96.8%) at a concentration of 0, 0.5, 1, 3, 4, 5, 7.5, 10 or 100 ppm (equal to 0, 0.04, 0.09, 0.27, 0.35, 0.44, 0.67, 0.89 and 8.96 mg/kg bw per day, respectively) for 90 consecutive days. Clinical observations, body weights and feed consumption were measured, and the eyes of all animals were examined by indirect ophthalmoscopy. Blood samples were taken for plasma tyrosine analysis, and overnight urine samples were collected for biochemical analyses. At the end of the scheduled period, the animals were killed and subjected to a limited postmortem examination. Selected organs were weighed, and specified tissues were taken for subsequent histopathological, electron microscopic or biochemical examinations.

There were no effects on mortality. Clinical signs of toxicity consisted of cloudy eyes at doses of 5 ppm and higher and red/brown staining on the tray paper of one 5 ppm cage; as it is consistent with the toxicity of mesotrione in other studies, this change was considered treatment related. Body weight was decreased at 100 ppm, but there were no clear effects on feed consumption. Under ophthalmological examination, changes to the cornea started at 7.5 ppm, with hazy to complete opacity of the cornea with or without vascularization. One animal in the 5 ppm dose group showed evidence of slight hazy opacity, but this was considered a random occurrence. Plasma tyrosine levels were increased at doses of 1 ppm and higher after 24 hours of treatment and at 0.5 ppm at weeks 1 and 14. There was an increase in urinary 4-hydroxyphenylpyruvate (HPPA), 4-hydroxyphenyllactate (HPLA) and 4-hydroxyphenylacetate (HPAA) phenyl acids in all tested animals at week 13, with a dose-related decrease in conjugates to free phenolic acids. There was a treatment-related increase in kidney weights at doses of 10 ppm and above and a treatment-related increase in liver weights at doses of 5 ppm and above. However, there were no gross pathological or histopathological changes noted in either organ at any dose in this study. Gross pathological changes were limited to increased opacity of the cornea at doses of 7.5 ppm and higher.

In the tissue biochemistry analysis, TAT was increased at doses of 3 ppm and higher, and HPPD was inhibited at doses of 0.5 ppm and above (Table 19).

In conclusion, in young male rats, there was a treatment-related inhibition of HPPD and increases in plasma tyrosine levels down to the lowest dose tested of 0.5 ppm. Adverse outcomes of tyrosinaemia were apparent at doses of 5 ppm and above, with increases in corneal opacity (Brammer, 1997d).

In a non-guideline dietary study to investigate dose–response relationships, 12 young adult female Alpk:APfSD (Wistar-derived) rats were fed a diet containing mesotrione (purity 96.8%) at a concentration of 0, 1, 5, 10, 50, 100, 1000 or 2500 ppm (equal to 0, 0.09, 0.48, 0.95, 4.82, 9.54, 94.83 and 236.75 mg/kg bw per day, respectively) for 90 consecutive days. An additional eight females per group were designated as satellites for interim kills on day 8 or 29. Clinical observations, body weights and feed consumption were measured, and the eyes of all animals were examined by indirect ophthalmoscopy. Overnight urine samples were collected for biochemical analyses. At the end of the scheduled period, the animals were killed, cardiac blood samples were taken for plasma tyrosine analysis and organs were removed, weighed and taken for biochemical analyses.

There were no effects on mortality. Clinical signs of toxicity were limited to cloudy eyes at doses of 1000 ppm and above. Body weights and feed consumption were decreased compared with controls in the 2500 ppm females, but unaffected at lower doses. Under ophthalmoscopic examination, there were increases in corneal opacity at doses of 100 ppm and higher and increases in vascularization and ghost vascularization at doses of 1000 ppm and above.

Table 19. Correlation of effects seen in rats administered mesotrione in the diet (maximum effect seen in each parameter)

	Dietary level of mesotrione (ppm)								
Parameter	**0**	**0.5**	**1**	**3**	**4**	**5**	**7.5**	**10**	**100**
Dose received (mg/kg bw per day)	–	0.04	0.09	0.27	0.35	0.44	0.67	0.89	8.96
Plasma tyrosine levels, week 13 (nmol/mL)	113	228*	431*	915**	1 241**	1 482**	1 934**	1 771**	2 772**
Kidney weight (% difference from control)	–	−1	1	1	1	4*	3	8**	8**
Liver weight (% difference from control)	–	−1	−1	4	5*	10**	12**	12**	15**
Corneal opacity (% affected)	0	0	0	0	0	6	25	31	94
Body weight, week 14 (% difference from control)	–	6	−1	3	0	4	−4	1	−4*
TAT activity (% difference from control)	100	110	125	151**	149**	148*	161**	140*	135
HPPD activity (% difference from control)	100	32	18*	20	18*	8*	10*	7*	3*
Urinary total phenolic acids (mg eq)	1.0	2.21	2.46	2.14	5.62	3.48	2.83	6.12	13.47

bw: body weight; eq: equivalents; HPPD: 4-hydroxyphenylpyruvate dioxygenase; ppm: parts per million; TAT: tyrosine aminotransferase; *: $P < 0.05$; **: $P < 0.01$ (Student's *t*-test, two-sided)

Source: Brammer (1997d)

Plasma tyrosine levels were increased to a statistically significant extent at doses of 5 ppm and higher and increased, but not statistically significantly, at 1 ppm. At doses of 1000 ppm and above, plasma tyrosine levels were between 1500 and 1600 nmol/mL, compared with control values of 112–127 nmol/mL, and remained relatively constant from weeks 1 to 13. At dose levels of 100 ppm and higher, there were increases in the levels of conjugated and free phenolic acids in the urine. As the dose increased, the proportion of free to conjugated acids decreased. The acids were identified as HPPA, HPLA and HPAA.

Liver weights were increased to a statistically significant extent at doses of 50 ppm and higher; however, the magnitude of the change was not biologically significant at any dose. There were no clear effects on kidney weights.

TAT induction occurred in all treated groups after the first week, but was statistically significant at doses of 5 ppm and above. By weeks 5 and 14, there was no change from control in TAT activity at doses of 100 ppm or less. HPPD inhibition occurred in all treated groups at all time points (Table 20).

In conclusion, in young female rats, inhibition of HPPD, a temporary activation of TAT and increases in plasma tyrosine levels occurred at doses of 1 ppm and higher. Adverse outcomes of tyrosinaemia occurred at doses of 100 ppm and above, with increases in corneal opacity (Brammer, 1997e).

In a non-guideline study investigating ocular toxicity development and reversibility, a group of 40 male Alpk:APfSD (Wistar-derived) rats were fed a diet containing 2500 ppm (equal to 272 mg/kg bw per day) mesotrione (purity 96.8%) for 35 consecutive days, with a concurrent control

Table 20. Correlation of effects seen in female rats administered mesotrione in the diet (maximum effect seen in each parameter)

	Dietary level of mesotrione (ppm)							
Parameter	**0**	**1**	**5**	**10**	**50**	**100**	**1 000**	**2 500**
Dose received (mg/kg bw per day)	–	0.09	0.48	0.95	4.82	9.54	94.83	236.75
Plasma tyrosine levels, week 13 (nmol/mL)	127	147	219**	249**	620**	836**	1 593**	1 534**
Liver weight, week 14 (% difference from control)	–	0	0	3	6	5	6*	7*
Corneal opacity (animals affected of 12)	0	0	0	0	0	2	11	10
Body weight, week 14 (% difference from control)	–	0	1	0	0	3	0	4*
TAT activity, week 2 (% of control)	100	132	165**	183**	200**	197**	233**	253**
HPPD activity, week 2 (% of control)	100	44**	12**	11**	12**	7**	2**	1**
Urinary total phenolic acids (mg eq)	0	0	0	0	0	1.82	7.0	23.0

bw: body weight; eq: equivalents; HPPD: 4-hydroxyphenylpyruvate dioxygenase; ppm: parts per million; TAT: tyrosine aminotransferase; *: $P < 0.05$; **: $P < 0.01$ (Student's *t*-test, two-sided)

Source: Brammer (1997e)

group consisting of 16 males fed untreated diet. Clinical observations, body weights and feed consumption were recorded, and eyes were examined by ophthalmoscopy. Blood samples were taken for measurement of plasma tyrosine levels, and eye and Harderian gland were taken for subsequent histopathological examination. At the end of the treatment period, all animals in the treated group without eye lesions and half of the animals in the control group were subjected to a postmortem examination. Approximately half of the animals that had eye lesions in the treated group were also given a postmortem examination, and the remainder were retained for a recovery period of 8 weeks prior to postmortem examination.

There were no effects on mortality. Body weight was decreased in the first 4 weeks of treatment, but not to a biologically significant degree. There were no changes to body weight thereafter.

Ophthalmological lesions were seen in treated animals starting at week 1 and were seen in 28/40 (70%) animals by the end of the treatment period. Two weeks into the recovery period, 9/15 (60%) animals exhibited corneal lesions. The vast majority of the changes at the end of the recovery period consisted of ghost vascularization.

Plasma tyrosine levels were increased starting at week 1 until week 14. The times for peak plasma tyrosine levels were weeks 2 and 6, with a substantial drop at week 7 and levels almost returning to control levels by week 14.

Microscopic changes in the eyes of animals killed at the end of the treatment period consisted of various severity grades of keratitis characterized by polymorphonuclear leukocytic infiltration of the outer corneal stroma with or without corneal epithelial disorganization and polymorphonuclear leukocytic infiltration of the corneal epithelium. The epithelial disorganization often took the form of a "V"-shaped area with basal cells present at the more superficial levels of the epithelium. Recovery animals showing no remaining changes at ophthalmoscopic evaluation showed no evidence of keratitis and no histopathological changes. Those recovery animals that exhibited ghost

vascularization at ophthalmoscopic evaluation exhibited histological evidence of remaining corneal vessels, and minimal or slight degrees of corneal fibroblasts were present in the subepithelial stroma. There was minimal or slight epithelial disruption in 3/15 rats. This was characterized by a slightly altered growth pattern of the epithelium so that the regular nature of the epithelial layers was disrupted. In some areas, there was hyaline material in a small number of basal epithelial cells.

In conclusion, corneal lesions associated with dietary administration of mesotrione for 5 weeks undergo ophthalmoscopic and histopathological recovery following cessation of treatment (Tinston, 1997).

In a non-guideline dietary study to investigate non-ocular end-points, 12 young adult male Alpk:APfSD rats per dose were fed diets containing mesotrione (purity 95.1%) at a concentration of 0, 10, 20, 50 or 125 ppm (equal to 0, 0.9, 1.7, 4.3 and 10.7 mg/kg bw per day, respectively) for 13 weeks. Body weight and feed consumption were monitored during this time. The eyes of all surviving animals were examined by ophthalmoscopy just prior to termination. At the end of the dosing period, the rats were killed, and blood samples were taken and stored. Liver and kidneys were weighed, and kidneys were processed to blocks and stored.

There were no treatment-related effects on mortality. Body weights were slightly decreased in the 125 ppm dose group at week 14, along with feed consumption from weeks 9 to 13. Corneal lesions occurred in all treated dose groups, with a dose-responsive increase in incidence. Although adjusted liver weights were increased in all treated groups, there was no dose–response relationship. Likewise, kidney weights were increased in all treated groups without a dose–response relationship.

In conclusion, under the limited investigations of this non-guideline study, body weight and feed consumption were the only signs of toxicity when ocular effects were excluded. These first signs of toxicity occurred at 125 ppm (equal to 10.7 mg/kg bw per day) (Brammer, 1995).

In a non-guideline dietary toxicity study to investigate reversibility, groups of 40 young adult male Alpk:APfSD (Wistar-derived) rats were fed diets containing mesotrione (purity 96.8%) at a concentration of 0 (two control groups), 5, 100 or 2500 ppm (equal to 0, 0.37, 7.52 and 192 mg/kg bw per day, respectively) for 90 consecutive days. These groups were subdivided into groups of eight rats and subjected to recovery periods of 0, 2, 4, 6 or 9 weeks for the 0 (first control group), 5 and 100 ppm dose groups and 0, 1, 2, 4 or 9 weeks for the 0 (second control group) and 2500 ppm dose groups. Clinical observations, body weights and feed consumption were measured, and the eyes of all animals were examined by indirect ophthalmoscopy. Blood samples were taken by tail venipuncture for plasma tyrosine analysis, and eight animals per group were killed at the end of each scheduled period and subjected to a postmortem examination. Additionally, cardiac blood samples were taken for plasma tyrosine analysis; liver and kidneys were weighed and were taken for subsequent examination by light and electron microscopy (control and 2500 ppm animals only) and for biochemical analyses.

There were no effects on mortality. Clinical signs of toxicity were limited to cloudy eyes in animals dosed at and above 5 ppm, and all signs of eye changes had resolved by week 17 in all dose groups. Body weights in animals dosed at 2500 ppm were decreased by 9% compared with controls during the last week of treatment and decreased by 10% in weeks 19–21 and by 8–10% at 23 weeks. Feed consumption was decreased sporadically in the 2500 ppm dose group during treatment. Effects in dose groups of 100 ppm and below were below levels considered adverse, although there was also evidence of recovery in the 5 and 100 ppm groups.

Under ophthalmoscopic examination, 63–70% of animals dosed at 100 and 2500 ppm exhibited corneal change by the end of the treatment period. Animals dosed at 5 ppm showed a lower incidence of lesions, including lower degrees of hazy or complete opacity with or without vascularization. Upon removal of mesotrione from the diet, corneal lesions began to reverse until only ghost vascularization persisted until the end of the recovery period.

Plasma tyrosine level was increased in 2500 ppm animals starting at 24 hours after the commencement of dosing and remained elevated until week 14. However, levels at 14 weeks were 1995 nmol/mL compared with 2917 nmol/mL, indicating a certain amount of adaptation. At the end of the recovery period, levels were within 21–32% of the control values, although the increase was statistically significant at week 23. At lower doses, plasma tyrosine concentrations were increased at week 1 and comparable with control values by week 18 (Table 21). Following homogenization of the liver and kidney samples, tyrosine concentrations were increased in the 5 and 100 ppm groups immediately following 90 days of treatment, but were comparable with control values following 9 weeks of recovery.

Table 21. Intergroup comparison of plasma tyrosine levels in rats

	Plasma tyrosine level (nmol/mL)				
	Part I			**Part II**	
Time point	**0 ppm (control)**	**5 ppm**	**100 ppm**	**0 ppm (control)**	**2 500 ppm**
24 hours	–	–	–	126	2 917**
Week 1	129.6	1 190.1**	2 021.6**	–	–
Week 14	155.4	1 283.4**	2 142.3**	192	1 995**
Week 15 + 1-week recovery	–	–	–	120	408**
Week 16 + 2-week recovery	105.1	128.1**	152.0**	113	423**
Week 18 + 4-week recovery	154.7	156.2	164.4	111	146
Week 20 + 6-week recovery	106.5	124.1*	129.6**	–	–
Week 23 + 9-week recovery	180.9	193.4	190.7	111	134**

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$ (Student's *t*-test, two-sided)

Source: Brammer (1997f)

In animals killed at the end of the treatment period, there were increases in liver and kidney weights following 90 days of mesotrione administration at doses of 5, 100 and 2500 ppm. Kidney and liver weights were increased compared with controls after 1 week of recovery at 2500 ppm, and liver weights were increased until and including 9 weeks of recovery at the same dose, although the magnitude of the increase was lower than immediately following treatment. In animals dosed at 5 and 100 ppm, there was no evidence of increased liver or kidney weights at 2–9 weeks of recovery.

TAT induction occurred at all doses during treatment and was similar to control values after 9 weeks of recovery. HPPD was inhibited following treatment at 5, 100 and 2500 ppm. There was recovery in the 2500 ppm group, although complete recovery did not seem to occur in the 5 and 100 ppm dose groups. However, there was a large variation in control values between the two parts of the study, and the finding is of limited value (Table 22).

In conclusion, mesotrione exhibited a potential for recovery from treatment-related effects on body weight and feed consumption, plasma and tissue tyrosine concentrations, enzyme induction and inhibition and ocular effects (Brammer, 1997f).

In a non-guideline dietary toxicity study in mice to investigate dose–response relationships, groups of 10 young adult C57BL/10JfAP/Alpk mice of each sex per dose were fed diets containing

Table 22. Intergroup comparison of HPPD activity in rats administered mesotrione in the diet

	HPPD activity (µL oxygen/min/mg protein)				
	Part I			Part II	
Week	0 ppm (control)	5 ppm	100 ppm	0 ppm (control)	2 500 ppm
14	0.880	0.099**	0.031**	0.141	0.005**
23 + 9-week recovery	1.030	0.664**	0.690**	0.207	0.161

HPPD: 4-hydroxyphenylpyruvate dioxygenase; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$ (Student's *t*-test, two-sided)

Source: Brammer (1997f)

mesotrione (purity 96.8%) at a concentration of 0, 1, 10, 50, 100, 350, 1000, 3500 or 7500 ppm (equal to 0, 0.16, 1.69, 8.49, 18.0, 58.5, 179.3, 599.9 and 1222.5 mg/kg bw per day for males and 0, 0.19, 1.94, 10.8, 20.5, 72.7, 214.9, 714.8 and 1436.4 mg/kg bw per day for females) for 90 consecutive days. An additional 10 males and 10 females per group were included as satellite groups for interim kills after 1 and 4 weeks of administration of mesotrione. Clinical observations, body weights and feed consumption were measured, and overnight urine samples were collected for biochemical analyses. At the end of the scheduled period, the animals were killed, cardiac blood samples were taken for plasma tyrosine analysis, liver and kidney were weighed, and samples were submitted for subsequent biochemical examinations and stored for possible histopathological examination.

There were no effects on mortality or clinical signs of toxicity. Body weights were decreased by up to 5% in males at week 8 and by up to 6% in females at week 7 at 7000 ppm. There were no effects on body weight at doses of 3500 ppm or lower. There were no effects on feed consumption at any dose tested.

Plasma tyrosine levels were increased at all time points in males and females at doses of 10 ppm and above. At doses of 50 ppm and higher in males, peak plasma tyrosine levels occurred at week 4. In the 1 ppm dose group, plasma tyrosine levels were increased in the first week following the start of treatment, but were comparable with control values thereafter in males. In females, plasma tyrosine levels were increased to a statistically significant extent at the 4-week time point, but were comparable with control values at weeks 1 and 14. In females at all doses, plasma tyrosine levels peaked at week 1 and, although still elevated compared with controls, decreased thereafter.

There was no evidence of conjugated phenolic acids in the urine of male or female mice at any dose. Free phenolic acids were increased in the urine of males at 10 ppm and above, but peaked at 100 ppm and decreased thereafter. In females, free phenolic acids were increased at all treatment doses, peaked at 100–350 ppm and decreased thereafter. The predominant phenolic acid in the urine samples was HPPA in both males and females, although HPLA and HPAA were also present.

There were no effects on liver or kidney weights.

TAT induction in male mice did not show consistent dose-responsiveness, but there was a trend towards increased induction from 50 to 1000 ppm at week 1, from 50 to 350 ppm at week 4 and from 100 to 3500 ppm at week 14. In females, there was a greater amount of statistical significance for this effect, as induction was increased at 50–3500 ppm at week 1, 1–7000 ppm at week 4 and 50–7000 ppm at week 14. HPPD inhibition was consistent in all males throughout the treatment period. In females, there was a statistically significant inhibition of HPPD at all doses in weeks 1 and 4 and at 100 ppm and above in week 14.

In conclusion, administration of mesotrione down to 1 ppm induced elevated plasma tyrosine levels in male and female mice. There was a correlation between the degree of tyrosinaemia, HPPD inhibition and excretion of phenolic acids in the urine in both sexes and a correlation with the induction of TAT in females. There were no significant clinical effects and no organ weight, gross

pathological or histopathological effects in the liver or kidney. There was a slight decrease in body weights in males and females at 7000 ppm (Brammer, 1997g).

In a non-guideline reproductive toxicity study in rats to investigate the effects of mesotrione in conjunction with dietary tyrosine, 20 time-mated female Alpk:APfSD (Wistar-derived) rats were fed diets containing 0 ppm mesotrione/0% tyrosine, 0 ppm mesotrione/0.5% tyrosine, 0 ppm mesotrione/1% tyrosine, 0 ppm mesotrione/2% tyrosine, 2500 ppm mesotrione/0% tyrosine, 2500 ppm mesotrione/0.5% tyrosine, 2500 ppm mesotrione/1% tyrosine or 2500 ppm mesotrione/2% tyrosine from day 1 of gestation until termination on day 29 postpartum. Clinical observations, body weights and feed consumption were recorded for the parent females. In addition, plasma tyrosine concentration was determined on two occasions. The females were allowed to litter, and the pups were sexed, counted, examined and weighed during the lactation period. Terminal blood samples were analysed for plasma tyrosine, and kidneys were examined for bilateral pelvic dilatation.

One dam dosed with 0 ppm mesotrione/2% tyrosine was found dead. There was a treatment-related increase in whole litter losses in dams dosed with 2500 ppm mesotrione and 0.5% tyrosine and above. All the dams in the 2500 ppm/2% tyrosine dose group were killed for humane reasons by day 11. Clinical signs of toxicity consisted of opaque eyes in all groups treated with mesotrione, but not in groups treated with tyrosine in the absence of mesotrione; hunched posture in at least one dam in each of the treated groups, but in two and five dams of the 2500 ppm mesotrione with 1% and 2% tyrosine groups, respectively; and piloerection in all of the groups treated with mesotrione and a slight increase in the dams treated with 2% tyrosine in the absence of mesotrione. During gestation, body weights were decreased in all dams given mesotrione without a dose–response trend dependent on the proportion of tyrosine. During lactation, body weights were decreased in dams given both mesotrione and tyrosine, with increased effects with increased tyrosine. Feed consumption was decreased in the first week of gestation in all groups given mesotrione and thereafter in the two groups given mesotrione and tyrosine. Plasma tyrosine levels were increased by an order of magnitude in all groups given mesotrione, with little effect of additional dietary tyrosine. In the groups given tyrosine in the absence of mesotrione, there was a slight increase in plasma tyrosine levels at 2% tyrosine at day 3 and at 1% and 2% tyrosine at day 51.

In the litters, pups showed an increase in opaque and cloudy eyes in all groups subjected to mesotrione, but in none of the groups exposed only to dietary tyrosine. The percentage of pups born live was decreased in the 2500 ppm mesotrione/1% tyrosine group, and the percentage of pups live at day 22 was decreased in both the 2500 ppm mesotrione/0.5% tyrosine and 2500 ppm mesotrione/1% tyrosine groups. Whole litter losses did not occur in the controls, but occurred at incidences of 1, 2 and 2 in the 0.5%, 1% and 2% dietary tyrosine groups and at incidences of 1, 6 and 10 in the 0%, 0.5% and 1% tyrosine with 2500 ppm mesotrione groups. Pup body weights were decreased by 7% in males and by 5% in females dosed with 2500 ppm mesotrione/1% tyrosine. Plasma tyrosine levels were increased in all dose groups; however, the increase was up to 1.8-fold in pups dosed with dietary tyrosine compared with increases of 16-fold in pups dosed with mesotrione or a combination of mesotrione and dietary tyrosine. There were no effects on the kidneys in pups dosed with dietary tyrosine, but there were increases in bilateral pelvic dilatation in all groups exposed to mesotrione and dietary tyrosine (36%, 45% and 52% in males and 37%, 45% and 33% in females at 0.5%, 1% and 2% tyrosine, respectively).

In conclusion, tyrosine and mesotrione caused increased plasma tyrosine levels and increases in whole litter losses, although the effect of mesotrione was greater than that of dietary tyrosine. Dietary tyrosine did not seem to have an effect on bilateral pelvic dilatation, whereas mesotrione with or without dietary tyrosine did increase kidney pathology and increase clinical signs in dams (Williams, 2000).

In a non-guideline developmental toxicity study to investigate the effects of tyrosinaemia on the ossification of the fetal skeleton and occurrence of abortions, 20 time-mated female New Zealand

White rabbits per dose were treated with diet containing 1% tyrosine from gestation day (GD) 8 to GD 21, 500 mg/kg bw per day mesotrione via gavage from GD 8 to GD 20 or 500 mg/kg bw per day mestrione via gavage from GD 8 to GD 20 along with 1% tyrosine in the diet from GD 8 to GD 21. A control group of 20 time-mated female New Zealand White rabbits was fed untreated diet and gavaged with water on GDs 8–20. The day of mating was designated day 1 of gestation. The rabbits were killed on day 30 of gestation. The following observations and measurements were made in the dams: clinical observations, body weights, feed consumption, ophthalmoscopy, plasma tyrosine concentration, HPPD and TAT activities in liver and kidney, postmortem examination (macroscopic), number of corpora lutea, gravid uterine weight and number and position of implantations in the uterus. The following observations and measurements were made in the fetuses/litters: number and position of live fetuses, number and position of intrauterine deaths (early and late), percentage preimplantation loss, percentage post-implantation loss, fetal weight, fetal sex, external and visceral variations and abnormalities and skeletal variations and abnormalities, including evaluation of bone and cartilage and ossification of the manus and pes.

There was one abortion in the mesotrione and tyrosine treatment following decreased feed consumption from day 17 and decreased body weight from day 19. One control female was found dead on day 19 (with live pups).

There were no biologically significant effects on adjusted mean body weights, and there was no evidence of effects on feed consumption. There were no changes at ophthalmoscopic examination.

Maternal plasma tyrosine levels increased starting at 12 hours post-dosing. At this point, dams dosed with tyrosine only had a 2.9-fold increase in tyrosine level, mesotrione-only dams had an 8-fold increase and dams dosed with both had a 17-fold increase. Values were similar at 12 hours post-dosing on day 14, but at 24 hours following dosing on day 14, tyrosine levels were increased by 1.5-, 2.6- and 3.8-fold in tyrosine-only dams, mesotrione-only dams and dams dosed with both tyrosine and mesotrione, respectively. Twenty-four hours following dosing on day 14, plasma tyrosine values were increased 1.5-fold in tyrosine-only dams, 2.6-fold in mesotrione-only dams and 3.8-fold in dams treated with both. By GD 29, plasma tyrosine values were practically comparable with control values. In the groups dosed with mesotrione and with mesotrione and tyrosine, there were comparable inhibitions of HPPD. In groups dosed with tyrosine alone, HPPD activity was comparable with control values. TAT activity was unaffected in all groups in the liver, but was inhibited in the kidneys in both mesotrione-dosed groups to similar extents. There were no macroscopic findings at necropsy. There were no treatment-related findings in the caesarean section parameters.

In the offspring, there was an increase in a minor defect of extra vessels arising from the aortic arch in both groups dosed with mesotrione; however, this type of finding was not seen in previous rabbit studies, and its relevance to treatment is unknown. In the skeletal examination, there were no major or minor defects, and changes were limited to delays in ossification. There was a distinct trend for delays to be more prevalent in groups exposed to both mesotrione and tyrosine over mesotrione alone and more prevalent in both compared with the tyrosine-only groups; however, delays occurred in all treated groups compared with controls.

In conclusion, although mesotrione had a greater effect than tyrosine, there was evidence that the effects were cumulative and a result of the increases in plasma tyrosine levels caused by the exposure to mesotrione. The delays in ossification were determined to be related to the increase in plasma tyrosine levels and a result of the inhibition of HPPD activity. The abortion in the mesotrione and tyrosine group was the only one in the study, and its relationship to treatment is unknown (Moxon, 2000).

(c) Studies on metabolites

Absorption, excretion and biotransformation

In a metabolism study on MNBA to investigate biotransformation in the rat, four male Alpk:APfSD rats were given a single oral [^{14}C]MNBA dose of 75 mg/kg bw. The excretion of radioactivity in urine and faeces was monitored for 12 hours after dosing. After this period, the rats

were killed, and the residual radioactivity was measured in the excreta, gastrointestinal tract, gastrointestinal tract contents and residual carcass. The metabolites present in urine and solvent extract of the gastrointestinal tract contents were identified and quantified by HPLC and HPLC with mass spectrometry.

Twelve hours following dosing, the largest proportion of the administered dose (43.6%) was found in the gastrointestinal tract, with 16.1% and 26.6%, respectively, found in the urine and faeces. Of the radioactivity recovered in the urine, 25% of the radioactivity recovered at 6 hours and 91% of the radioactivity recovered at 12 hours were characterized as AMBA. At 12 hours following dosing, 100% of the radioactivity in the gastrointestinal tract was characterized as AMBA. According to the study author, the molecular weights of both MNBA and AMBA are lower than the biliary elimination cut-off, and so it is assumed that radioactivity in faeces represents unabsorbed dose.

In conclusion, MNBA is minimally absorbed and excreted in the urine. The majority is converted to AMBA in the gut, where it is excreted unabsorbed (Gledhill, 2000).

Acute studies on metabolites

Acute toxicity studies on the metabolites are summarized in Table 23.

Table 23. Acute toxicity of mesotrione metabolites

Test substance	Species	Strain	Sex	Route	Purity (%)	Result	Reference
MNBA	Rat	Alpk:APfSD (Wistar-derived)	Male and female	Oral	97	LD_{50} > 5 000 mg/kg bw	Robinson (1996)
AMBA	Rat	Alpk:APfSD (Wistar-derived)	Male and female	Oral	99	LD_{50} > 5 000 mg/kg bw	Lees (1996)

AMBA: 4-(methylsulfonyl)-2-aminobenzoic acid; bw: body weight; LD_{50}: median lethal dose; MNBA: 2-nitro-4-(methylsulfonyl)-benzoic acid

Short-term studies of toxicity on metabolites

In a short-term oral toxicity study, groups of five young adult Alpk:APfSD (Wistar-derived) rats of each sex per dose were given MNBA (purity 97.1%) in corn oil via gavage at 0, 15, 150 or 1000 mg/kg bw per day for 28 consecutive days. Clinical observations, body weights and feed consumption were measured throughout the study. In the fourth week of the study, the following tests were assessed: sensory reactivity to stimuli, grip strength and motor activity. At the end of the scheduled period, the animals were killed and subjected to a postmortem examination. Cardiac blood samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

There was no treatment-related mortality, and there were no clinical signs of toxicity. There were no effects on body weight, feed consumption, detailed clinical parameters or functional observational battery parameters. There were no effects on haematological parameters, and the only change in clinical chemistry was a slight, non-adverse decrease in alanine aminotransferase activity. Spleen weights were decreased by 12% in high-dose females, and testes weights were increased by 13% in high-dose males. However, as there were no findings in the gross necropsy or histopathological examination, the changes were of unknown adversity.

The NOAEL for MNBA was 1000 mg/kg bw per day, the highest dose tested (Milburn, 1998).

In a short-term oral toxicity study, groups of 12 young adult Alpk:APfSD (Wistar-derived) rats of each sex per dose were given diets containing MNBA (purity 98.3%) at 0, 100, 650 or 3000 ppm (equal to 0, 7.7, 50.6 and 231.0 mg/kg bw per day for males and 0, 8.8, 56.9 and 263.7 mg/kg bw

per day for females, respectively) for 90 consecutive days. Clinical observations, body weights and feed consumption were measured throughout the study, and urine samples were taken for clinical pathology during week 13 of the study. In addition, detailed clinical observations, including quantitative assessments of landing foot splay, sensory perception and muscle weakness, and assessment of motor activity were performed during week 12 of the study. At the end of the scheduled period, the animals were killed and subjected to a postmortem examination. Cardiac blood samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

There were no mortalities or treatment-related clinical effects. There was an 8% decrease in body weight in high-dose males and a sporadic, non-adverse decrease in feed consumption at the same dose. There were no changes to ophthalmological parameters and no changes in the detailed observations of the functional observational battery. There were no changes in haematological parameters. There were 1.2- and 1.9-fold increases in plasma tyrosine levels in males only at 650 and 3000 ppm, respectively; however, the increase was not associated with any adverse changes in the animals. In females, triglyceride levels were increased 36% over those of concurrent controls in the 3000 ppm dose group. There were no changes in urine analysis parameters, and no phenolic acids were detected in the urine. There were no changes noted at necropsy or in the histopathological examination.

The LOAEL for MNBA was 3000 ppm (equal to 231.0 mg/kg bw per day), based on decreased body weight in males and increased triglyceride levels in females. The NOAEL was 650 ppm (equal to 50.6 mg/kg bw per day) (Rattray, 2000).

Genotoxicity studies on metabolites

Genotoxicity studies on mesotrione metabolites are summarized in Table 24.

Special studies on metabolites

In a non-guideline study to investigate the inhibition of HPPD by MNBA, excised livers from untreated male Alpk:APfSD rats were exposed to 0.02 or 20 µmol/L of MNBA as well as 0.02 and 20 µmol/L concentrations of mesotrione and 2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) (known inhibitors of HPPD).

The positive control substances, mesotrione and NTBC, inhibited HPPD at both 0.02 and 20 µmol/L. At the lower dose, mesotrione inhibited HPPD by 78%, and NTBC inhibited HPPD by 70.3%. For MNBA, there was no inhibition at 0.02 µmol/L, and HPPD was inhibited by 7.2% at 20 µmol/L.

In conclusion, MNBA is a very weak inhibitor of HPPD compared with mesotrione and NTBC (Elcombe & Meadowcroft, 1998a).

In a non-guideline study to investigate the inhibition of HPPD by AMBA, excised livers from untreated male Alpk:APfSD rats were exposed to 0.02 or 20 µmol/L of AMBA as well as 0.02 and 20 µmol/L concentrations of mesotrione and NTBC (known inhibitors of HPPD).

The positive control substances, mesotrione and NTBC, inhibited HPPD at both 0.02 and 20 µmol/L. At the lower dose, mesotrione inhibited HPPD by 78%, and NTBC inhibited HPPD by 70.3%. For AMBA, there was no inhibition at 0.02 µmol/L, and HPPD was inhibited by 18.7% at 20 µmol/L.

In conclusion, AMBA is a very weak inhibitor of HPPD compared with mesotrione and NTBC (Elcombe & Meadowcroft, 1998b).

Table 24. Genotoxicity studies with mesotrione metabolites

Test substance	End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro						
MNBA	Reverse mutation	*Salmonella typhimurium* and *Escherichia coli*	0, 100, 200, 500, 1 000, 2 500 or 5 000 µg/plate (±S9)	97	Negative	Callander (1996a)
MNBA	Mammalian cell cytogenetics	Human lymphocytes	0, 250 or 2 451 µg/mL (+S9)	98.8	Negative	Fox (2000a)
			0, 250, 1 000/1 500 or 2 000/2 451 µg/mL (–S9)		Negative	
AMBA	Reverse mutation	*S. typhimurium* and *E. coli*	0, 100, 200, 500, 1 000, 2 500 or 5 000 µg/plate (±S9)	99	Negative	Callander (1996b)
AMBA	Mammalian cell cytogenetics	Human lymphocytes	250, 1 000 or 2 150 µg/mL (+S9)	100	Negative	Fox (2000b)
			250, 1 000 or 2 150 µg/mL (–S9)		Positive	
In vivo						
MNBA	Rat liver unscheduled DNA synthesis	Alpk:APfSD rat liver, males	1 000 or 2 000 mg/kg bw Harvest time: 2, 6 or 16 h	98.8	Negative	Clay (2000)
MNBA	Rat micronucleus	Alpk:APfSD rat bone marrow, males	0 or 2 000 mg/kg bw Harvest time: 28 and 48 h	98.9	Negative	Fox (2000c)

AMBA: 4-(methylsulfonyl)-2-aminobenzoic acid; bw: body weight; DNA: deoxyribonucleic acid; MBNA: 2-nitro-4-(methylsulfonyl)-benzoic acid; S9: 9000 × *g* supernatant fraction from liver homogenate from Aroclor-treated rats

3. Observations in humans

In an acute oral capsule human volunteer study to identify suitable urinary markers for worker exposure, three groups of six male human volunteers were administered a single oral dose of 0.1, 0.5 or 4 mg/kg bw. Volunteers were fed controlled diet to provide a consistent intake of tyrosine 72 hours prior to dosing and in the 96-hour period following dosing. Plasma and urine samples were collected in the predosing and post-dosing intervals and were analysed for tyrosine and test substance. Samples from the volunteers given a 4 mg/kg bw dose were monitored for tyrosine metabolites. Volunteers were examined for changes to ophthalmoscopy, clinical chemistry, haematology and urine analysis, and vital signs and symptomatology were monitored post-dosing.

Plasma tyrosine levels peaked within 24 hours of administration in all three dose groups. In the 0.1 and 0.5 mg/kg bw dose groups, plasma tyrosine levels had returned to predosing levels at the end of the 24-hour period. In the 4 mg/kg bw group, plasma tyrosine levels had returned to predosing levels by the 48-hour time period. Peak tyrosine concentrations occurred between 91.0 and 160, 121 and 210, and 241 and 420 nmol/mL plasma in the 0.1, 0.5 and 4 mg/kg bw groups, respectively. There was no statistical difference in elevations in the 0.1 and 0.5 mg/kg bw groups. In comparison, in a study with a strong HPPD inhibitor, NTBC, plasma tyrosine levels peaked at a mean of 1155 ± 121.2 nmol/mL following a single NTBC administration of 1 mg/kg bw (Stevens, 1998). There were no adverse signs noted, and peak concentrations of test substance were reached within 1 hour of dosing. A significant proportion of the administered dose was excreted in the urine as test substance, whereas tyrosine metabolites were detected in the urine following dosing with mesotrione at 4 mg/kg bw (Hall, 1998a).

In an acute dermal spray human volunteer study to determine urinary concentrations of mesotrione and effects on plasma tyrosine level following dermal exposure, three groups of six male human volunteers were administered single dermal doses of 5 µg of 100 g/L mesotrione per square centimetre (group 1), 5 µg of 480 g/L mesotrione per square centimetre (group 2) or 32 µg of 480 g/L mesotrione per square centimetre (group 3), representing two formulations and two concentrations of the 480 g/L formulation. The test substance was applied to a total area of 800 cm^2 and left unoccluded for 10 hours before the site was washed. Tape stripping of the washed sites was performed on a 6 cm^2 area in three sessions: post-initial washing, after 24 hours of wearing a T-shirt and following showering. Plasma and urine samples were collected in the predosing and post-dosing intervals and analysed for tyrosine and test substance. Urine samples were monitored for known tyrosine metabolites. Volunteers were examined for changes to ophthalmoscopy, clinical chemistry, haematology and urine analysis, the test site was monitored for signs of irritation, and vital signs and symptomatology were monitored post-dosing.

Dermal reactions consisted of an increase in mild transient itching in participants in group 3, a mild, transient burning sensation 30 minutes after application in one participant in group 1 and a mild, transient stinging sensation 9 hours following application in one participant in group 2. Erythema was noted in all groups, although the study authors attributed the reaction to the procedure or tape stripping. Symptomatology designated as "possibly related" to administration of the test substance consisted of mild tingling at the dose site 9 hours following application in one group 1 member and mild headache experienced 4 hours following application in one group 3 member.

There were no changes in plasma tyrosine level in any of the groups and no markers of mesotrione in the urine in the two lower-dose groups. In group 3, mesotrione was detected at slightly above the level of quantification in five out of six volunteers in up to five of the 13 samples collected from each participant. There were no quantifiable concentrations of mesotrione isolated in the plasma in any of the groups. Tape stripping indicated that mesotrione was located in the stratum corneum following initial washing, but was removed by washing and/or contact with clothing soon thereafter (Hall, 1998b).

Comments

Biochemical aspects

Excretion and tissue retention studies were performed in mice and rats. In addition, a full set of metabolism studies was performed in rats. Radiolabelled mesotrione was administered by gavage in both species. In mice, mesotrione was extensively absorbed (> 60%) and primarily excreted in the urine, constituting 41–59% of the administered low dose (1 mg/kg bw) and 63–70% of the high dose (100 mg/kg bw). Faecal elimination comprised 21–38% of the administered dose. Elimination was essentially complete within the first 24 hours; by 72 hours following dosing, elimination comprised 79–95% of the administered dose. In rats, mesotrione was rapidly and extensively absorbed (> 60%), metabolized to a limited extent and excreted primarily in the urine after single low (54–56% at 1 mg/kg bw) or high doses (62–63% at 100 mg/kg bw) or repeated low doses (61–67% at 1 mg/kg bw per day) over 14 days to rats. Biliary excretion was minimal. Most of the radioactivity was excreted as the parent compound within the first 12 hours post-dosing. Highest levels were found in liver, kidneys and gastrointestinal tract in both species, with 10–12% present in tissues following a low dose and less than 0.3% following a high dose.

In studies performed only in rats, there was no evidence of accumulation. C_{max} values were 0.27 and 0.25 µg eq/g in male and female rats, respectively, at the low dose (1 mg/kg bw) and 40.4 and 19.9 µg eq/g, respectively, at the high dose (100 mg/kg bw). The T_{max} was 0.5 hour at the low dose and 1.5 hours at the high dose. Half-lives in blood were less than 2 hours, regardless of sex or dose. There were no notable differences in absorption or excretion between the sexes. Mesotrione and its metabolites were not excreted in expired air. Parent compound accounted for more than 43–64% of the administered dose in the urine and 0–8% of the administered dose in the faeces.

In rats, the metabolites produced from hydroxylation of the dione ring include 4-hydroxy-mesotrione, 5-hydroxy-mesotrione, MNBA and AMBA. There is also a proposed cleavage of the molecule into constituent rings and reduction by the gut microflora, resulting in a number of unidentified metabolites, accounting for a total of approximately 0–12% of the administered dose in the faeces.

Toxicological data

In the rat, mesotrione is of low acute oral toxicity (LD_{50} > 5000 mg/kg bw), low acute dermal toxicity (LD_{50} > 2000 mg/kg bw) and low acute inhalation toxicity (LC_{50} > 4.75 mg/L). In the rabbit, mesotrione was non-irritating to the skin and mildly irritating to the eyes. Mesotrione was not a dermal sensitizer in guinea-pigs (maximization test).

The primary effect of mesotrione in mammals is the inhibition of HPPD, a key enzyme of the tyrosine catabolic pathway. Inhibition of HPPD by mesotrione results in raised plasma tyrosine levels, which appear to be responsible for the critical effects observed (ocular, kidney, liver and thyroid toxicity). The plateau levels of plasma tyrosine after mesotrione administration are higher in rats (males > females) than in mice. The difference in sensitivity between male and female rats as well as between rats and mice can be attributed to differences in tyrosine catabolism. If the activity of TAT is low, as it is in the male rat, tyrosine cannot convert quickly to HPPA; when HPPD is inhibited, the resultant increase in plasma tyrosine levels leads to toxicity.

The critical effect (ocular toxicity) associated with the administration of mesotrione is mediated by these increased systemic levels of tyrosine and occurs only when plasma tyrosine levels exceed about 1000 nmol/mL. The ocular sensitivity of the various species to tyrosine plasma levels seems to be similar; the difference in overall toxicity of mesotrione among the species is attributable to the different levels of plasma tyrosine achieved after HPPD inhibition by mesotrione.

Although the rat is the most sensitive species for assessing tyrosine-mediated mesotrione toxicity, the mouse is a better model for such effects in humans. Humans and mice have similar TAT activities and do not experience the adverse effects associated with the same degree of HPPD inhibition in rats. The effects on the eyes, kidneys, liver and thyroid seen in the rat are unlikely to occur in humans exposed to mesotrione owing to differences in tyrosine metabolism. As all the relevant studies normally performed in the rat were also performed in the mouse, it was determined that the risk assessment would be based on toxicity in the mouse, rabbit and dog.

In a 90-day oral toxicity study in mice, animals were given diets containing mesotrione at a concentration of 0, 10, 50, 350 or 7000 ppm (equal to 0, 1.7, 8.4, 61.5 and 1212.4 mg/kg bw per day for males and 0, 2.4, 12.4, 80.1 and 1537.1 mg/kg bw per day for females, respectively). The NOAEL was 7000 ppm (equal to 1212.4 mg/kg bw per day), the highest dose tested.

In a 90-day oral toxicity study in rats, animals were given diets containing mesotrione at a concentration of 0, 1, 125, 1250 or 12 500 ppm (equal to 0, 0.09, 10.96, 112.09 and 1110.86 mg/kg bw per day for males and 0, 0.10, 12.81, 125.58 and 1212.53 mg/kg bw per day for females, respectively). At 125 ppm (equal to 10.96 mg/kg bw per day), male rats showed evidence of increased corneal opacity and vascularization and decreased body weight and feed efficiency.

In a 13-week oral toxicity study in rats, animals were given diets containing mesotrione at a concentration of 0, 2.5, 5.0, 7.5 or 150 ppm (equal to 0, 0.21, 0.41, 0.63 and 12.46 mg/kg bw per day for males and 0, 0.23, 0.47, 0.71 and 14.48 mg/kg bw per day for females, respectively). There were no non-ocular findings in either male or female rats in this study. At 7.5 ppm and above, males showed evidence of cloudy eyes.

In a 13-week oral capsule toxicity study in dogs, animals were exposed to 0, 100, 600 or 1000 mg/kg bw per day. At 1000 mg/kg bw per day, body weights were decreased in males compared with controls and there was an increase in minimal/slight focal mesothelial proliferation of the atrium of the heart in two males. The NOAEL was 600 mg/kg bw per day.

In a 1-year oral capsule toxicity study in dogs, animals were exposed to 0, 10, 100 or 600 mg/kg bw per day. At the high dose, body weights were decreased in females, and lenticular opacity was observed in one male and one female. In the male, the lenticular opacity was associated with unilateral keratitis and periorbital haemorrhage; in the female, it was associated with unilateral corneal erosion. The NOAEL was 100 mg/kg bw per day.

In a 1-year oral toxicity study in mice, animals were given diets containing mesotrione at a concentration of 0, 10, 50, 350 or 7000 ppm (equal to 0, 1.5, 7.8, 56.2 and 1114 mg/kg bw per day for males and 0, 2.1, 10.3, 72.4 and 1495 mg/kg bw per day for females, respectively). At the highest dose tested, males exhibited decreased body weight and body weight gains. There were no effects in females at the highest dose tested. The NOAEL was 350 ppm (equal to 56.2 mg/kg bw per day).

In an 18-month oral toxicity and carcinogenicity study in mice, animals were given diets containing mesotrione at a concentration of 0, 10, 350 or 3500/7000 ppm (equal to 0, 1.4, 49.7 and 897.7 mg/kg bw per day for males and 0, 1.8, 63.5 and 1103 mg/kg bw per day for females, respectively). As seen in the 1-year study, body weight, body weight gains and feed efficiency were decreased in males at the highest dose tested, and there were no effects in females at the highest dose tested. There was no evidence of carcinogenicity. The NOAEL was 350 ppm (equal to 49.7 mg/kg bw per day).

In a 2-year carcinogenicity study in rats, animals were given diets containing mesotrione at a concentration of 0, 7.5, 100 or 2500 ppm (equal to 0, 0.48, 6.48 and 159.9 mg/kg bw per day for males and 0, 0.57, 7.68 and 189.5 mg/kg bw per day for females, respectively). There was no evidence of carcinogenicity. In males, changes at all doses consisted of cloudy eyes, corneal opacities, vascularization and keratitis in the clinical, ophthalmological and histopathological examinations, decreased body weights, hepatocyte fatty vacuolation in the liver and thyroid follicular cysts.

The Meeting concluded that mesotrione is not carcinogenic in mice or rats.

Mesotrione was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. There was no evidence of genotoxicity.

The Meeting concluded that mesotrione is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that mesotrione is unlikely to pose a carcinogenic risk to humans.

In a two-generation reproductive toxicity study in mice, animals were given diets containing mesotrione at a concentration of 0, 10, 50, 350, 1500 or 7000 ppm (equal to 0, 2.1, 10.2, 71.4, 311.8 and 1472 mg/kg bw per day for males and 0, 2.1, 10.0, 71.3, 301.6 and 1439 mg/kg bw per day for females, respectively). At the highest dose tested, F_1 adults and pups showed evidence of cataractous changes at clinical, gross and histopathological examination. Pups at the next lower dose also exhibited decreased body weight and body weight gain, clinical, gross and histopathological changes to the eyes (opaque/cloudy eyes, cataractous change) and increased plasma tyrosine levels. The NOAEL for parental toxicity was 1500 ppm (equal to 301.6 mg/kg bw per day). The NOAEL for reproductive toxicity was 7000 ppm (equal to 1439 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 350 ppm (equal to 71.3 mg/kg bw per day).

In a three-generation reproductive toxicity study in rats, animals were given diets containing mesotrione at a concentration of 0, 2.5, 10, 100 or 2500 ppm (equal to 0, 0.3, 1.1, 11.6 and 278.1 mg/kg bw per day for males and 0, 0.3, 1.1, 11.7 and 297.2 mg/kg bw per day for females, respectively), with an F_2 recovery group in which the dams were not treated through gestation. Effects in the parental generations consisted of ocular changes in clinical, ophthalmological, gross and histopathological examinations at dietary concentrations of 10 ppm and above, along with increased plasma tyrosine levels. In pups, cloudy/opaque eyes, keratitis and/or corneal vascularization were observed in all treated groups in males and at 100 and 2500 ppm in females in litters exposed to mesotrione in utero. Plasma tyrosine levels were measured in pups in the F_{3A} groups and were increased in all treatment groups in the continuous treatment animals; levels were comparable with

those of controls in all the recovery groups. Decreased litter size, decreased survival, decreased percentage of pups born live and increased whole litter loss were observed at the highest dose tested.

A mode of action study in rats was performed to determine the link between tyrosinaemia and the changes noted in the rat reproductive toxicity study. In a modified one-generation reproductive toxicity study, animals were exposed to 0 ppm mesotrione with 0%, 0.5%, 1% or 2% tyrosine or to 2500 ppm mesotrione with 0%, 0.5%, 1% or 2% tyrosine from day 1 of gestation until termination on day 29 postpartum. Tyrosine and mesotrione increased plasma tyrosine levels and caused increases in whole litter losses, although the effect of mesotrione was greater than that of dietary tyrosine. The Meeting concluded that the reproductive effects observed in rats were likely a consequence of the elevated levels of tyrosine.

In a developmental toxicity study in mice, pregnant females were dosed at 0, 10, 60, 150 or 600 mg/kg bw per day. There were no signs of maternal or embryo/fetal toxicity up to 600 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study in rats, pregnant females were dosed at 0, 100, 300 or 1000 mg/kg bw per day. Maternal body weight and feed consumption were decreased at all doses. In fetuses, delays in ossification were increased at all doses.

In a developmental toxicity study in rabbits, pregnant females were dosed at 0, 100, 250 or 500 mg/kg bw per day. At 250 and 500 mg/kg bw per day, there were increases in abortions and decreased defecation. The NOAEL for maternal toxicity was 100 mg/kg bw per day, based on increased abortions and decreased defecation at 250 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 500 mg/kg bw per day, the highest dose tested.

An investigative study was performed with pregnant female rabbits treated as follows: control (no tyrosine or mesotrione), tyrosine (1% dietary), mesotrione (500 mg/kg bw per day by gavage) and tyrosine and mesotrione (1% dietary tyrosine and 500 mg mesotrione/kg bw per day by gavage). Plasma tyrosine levels were increased in all groups treated with mesotrione, tyrosine or a combination of the two. In groups treated with both mesotrione and tyrosine, the plasma tyrosine levels were highest, followed by mesotrione-only treated dams and, lastly, tyrosine-only treated dams. Likewise, delays in ossification were most prevalent in the fetuses of dams treated with both mesotrione and tyrosine, followed by mesotrione-only and tyrosine-only treated dams; however, delays were prevalent in all treated groups at rates higher than those in the concurrent controls. There was only one abortion, which occurred in the group treated with both mesotrione and tyrosine. As such, the Meeting concluded that delays in ossification were related to the increase in plasma tyrosine levels. There was insufficient information to enable a conclusion to be reached with regard to abortions.

The Meeting concluded that mesotrione is not teratogenic.

In an acute neurotoxicity study in rats, no neurotoxic effects were seen at 2000 mg/kg bw, the highest dose tested.

In a 13-week dietary neurotoxicity study in rats, ophthalmoscopic findings were observed at 100 ppm (equal to 8.25 mg/kg bw per day). No neurotoxicity was observed up to 5000 ppm (equal to 402.8 mg/kg bw per day), the highest dose tested.

The Meeting concluded that mesotrione is not neurotoxic.

Toxicological data on metabolites and/or degradates

For MNBA, a plant and livestock metabolite, studies of metabolism, acute toxicity, short-term toxicity, genotoxicity and HPPD inhibition were performed.

When given to rats as a single oral dose of 75 mg/kg bw, [^{14}C]MNBA was minimally absorbed and excreted in the urine. The majority was converted to AMBA in the gut, which was excreted unabsorbed.

MNBA is of low acute oral toxicity, with an LD_{50} of greater than 5000 mg/kg bw.

In a 28-day gavage study in rats, MNBA was given in corn oil at a dose of 0, 15, 150 or 1000 mg/kg bw per day. The NOAEL was 1000 mg/kg bw per day, the highest dose tested.

In a 90-day study in rats, animals were given MNBA in the diet at a concentration of 0, 100, 650 or 3000 ppm (equal to 0, 7.7, 50.6 and 231.0 mg/kg bw per day for males and 0, 8.8, 56.9 and 263.7 mg/kg bw per day for females, respectively). At 3000 ppm, body weights were decreased statistically significantly (by 8%) in males, and triglyceride levels were increased (by 36%) in females. The NOAEL was 650 ppm (equal to 50.6 mg/kg bw per day), based on equivocal effects on body weight and increased triglyceride levels.

MNBA was tested in an adequate range of genotoxicity assays. No evidence of genotoxicity was observed.

MNBA was a very weak inhibitor of HPPD compared with mesotrione.

For AMBA, a plant and livestock metabolite, studies of acute toxicity, genotoxicity and HPPD inhibition were performed.

AMBA is of low acute oral toxicity, with an LD_{50} of greater than 5000 mg/kg bw.

AMBA showed no evidence of genotoxicity in a reverse mutation assay or in a mammalian cell cytogenetic assay in the presence of metabolic activation and gave positive results in the mammalian cell cytogenetic assay in the absence of metabolic activation.

AMBA was a very weak inhibitor of HPPD compared with mesotrione.

As there was insufficient information to determine the toxicological profile of MNBA and AMBA, their toxicological relevance was assessed using JMPR's metabolite assessment scheme included in the guidance document for WHO monographers.[1] On the basis of this assessment, the Meeting concluded that these metabolites are unlikely to be a safety concern.

Human data

In a study in which human volunteers were exposed to a single oral dose of mesotrione of 0.1, 0.5 or 4 mg/kg bw in capsules, there were no symptoms, clinical signs or changes on ophthalmological examination. In volunteers given 4 mg/kg bw, plasma tyrosine levels were increased up to 48 hours following dosing, with a peak tyrosine concentration of up to 420 nmol/mL plasma; unchanged mesotrione was found in the urine.

There are no reports of poisoning cases with mesotrione.

The Meeting concluded that the existing database on mesotrione was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.5 mg/kg bw on the basis of the NOAEL of 49.7 mg/kg bw per day, based on decreased body weight, body weight gain and feed efficiency in male mice in an 18-month toxicity and carcinogenicity study. A safety factor of 100 was applied.

The Meeting considered the mode of action of the HPPD-dependent effects of mesotrione and concluded that the rat was not an appropriate model on which to base the toxicological evaluation.

The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for mesotrione in view of its low acute oral toxicity and the absence of developmental toxicity and any other toxicological effects that would be likely to be elicited by a single dose.

[1] http://www.who.int/entity/foodsafety/areas_work/chemical-risks/jmpr_Guidance_Document_FINAL.pdf

Levels relevant to risk assessment of mesotrione

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	350 ppm, equal to 49.7 mg/kg bw per day	3 500/7 000 ppm, equal to 897.7 mg/kg bw per day
		Carcinogenicity	3 500/7 000 ppm, equal to 897.7 mg/kg bw per day[b]	–
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	7 000 ppm, equal to 1 439 mg/kg bw per day[b]	–
		Parental toxicity	1 500 ppm, equal to 301.6 mg/kg bw per day	7 000 ppm, equal to 1439 mg/kg bw per day
		Offspring toxicity	350 ppm, equal to 71.3 mg/kg bw per day	1 500 ppm, equal to 301.6 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	600 mg/kg bw per day[b]	–
		Embryo and fetal toxicity	600 mg/kg bw per day[b]	–
Rat	Two-year study of toxicity and carcinogenicity[a]	Carcinogenicity	159.9 mg/kg bw per day[b]	–
Rabbit	Developmental toxicity study[c]	Maternal toxicity	100 mg/kg bw per day	250 mg/kg bw per day
		Embryo and fetal toxicity	500 mg/kg bw per day[b]	—
Dog	One-year study of toxicity[d]	Toxicity	100 mg/kg bw per day	600 mg/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.
[d] Capsule administration.

Estimate of acceptable daily intake (ADI)

0–0.5 mg/kg bw

Estimate of acute reference dose (ARfD)

Unnecessary

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to mesotrione

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; extensive (> 60%)
Dermal absorption	No data
Distribution	Rapidly eliminated; highest residues in carcass, gastrointestinal tract, liver and kidneys
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Largely complete within 24 hours; primarily via urine (41–70% in mice and 54–84% in rats), with 21–38% in faeces (rats and mice)
Metabolism in animals	Mostly excreted unchanged
Toxicologically significant compounds in animals and plants	Mesotrione, MNBA and AMBA
Acute toxicity	
Rat, LD_{50}, oral	> 5 000 mg/kg bw
Rat, LD_{50}, dermal	> 2 000 mg/kg bw
Rat, LC_{50}, inhalation	> 4.75 mg/L
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Mildly irritating
Guinea-pig, dermal sensitization	Not sensitizing (maximization test)
Short-term studies of toxicity	
Target/critical effect	Body weight
Lowest relevant oral NOAEL	100 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	1 000 mg/kg bw per day (rabbit)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Body weight
Lowest relevant oral NOAEL	49.7 mg/kg bw per day (mouse)
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans
Genotoxicity	
	Unlikely to be genotoxic
Reproductive toxicity	
Target/critical effect	Decreased body weight, clinical, gross and histopathological changes to the eye
Lowest relevant parental NOAEL	301.6 mg/kg bw per day (mouse)
Lowest relevant offspring NOAEL	71.3 mg/kg bw per day (mouse)
Lowest relevant reproductive NOAEL	1 439 mg/kg bw per day (mouse)
Developmental toxicity	
Target/critical effect	Abortions and decreased faecal output
Lowest relevant maternal NOAEL	100 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	500 mg/kg bw per day, highest dose tested (rabbit)

Neurotoxicity	
Acute neurotoxicity NOAEL	2 000 mg/kg bw, highest dose tested
Subchronic neurotoxicity NOAEL	402.8 mg/kg bw per day, highest dose tested
Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Studies on toxicologically relevant metabolites	*MNBA*: Metabolism: Minimally absorbed, excreted primarily in urine, majority in gut at 12 hours converted to AMBA HPPD inhibition: very weak compared with mesotrione Oral LD_{50}: > 5 000 mg/kg bw NOAEL: 1 000 mg/kg bw per day, highest dose tested (4-week gavage study in rats) NOAEL: 50.6 mg/kg bw per day, based on equivocal decreases in body weight and increased triglyceride levels (90-day study in rats) Unlikely to be genotoxic *AMBA*: HPPD inhibition: very weak compared with mesotrione Oral LD_{50}: > 5 000 mg/kg bw Unlikely to be genotoxic
Medical data	
	No studies submitted

Summary

	Value	Study	Safety factor
ADI	0–0.5 mg/kg bw	Eighteen-month study of toxicity and carcinogenicity (mouse)	100
ARfD	Unnecessary	–	–

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Appendix 1: Mode of action

The following analysis is based on the methodology developed by an ILSI/HESI workgroup and is based on the decision logic outlined by Seed et al. (2005). The human relevance framework is based on a four-part analysis:

1) Is the weight of evidence sufficient to establish the MOA in animals?
2) Are the key events in the animal MOA plausible in humans?
3) Taking into account kinetic and dynamic factors, is the animal MOA plausible in humans?
4) Statement of confidence; analysis; implications.

Mesotrione has been reviewed below, using the human relevance framework principles.

Is the weight of evidence sufficient to establish the MOA in animals?

Inhibition of HPPD

Mesotrione is a triketone herbicide and exerts its MOA via inhibition of the enzyme HPPD (Lee et al., 1997). HPPD occurs in plants and animals, the 52 active site amino acid residues being similar across phyla and highly conserved within mammalian species (Table A-1).

Table A-1. HPPD amino acid sequence comparisons across phyla

	Arabidopisis	**Maize**	**Rat**	**Mouse**	**Pig**	**Human**
Arabidopisis		60%[1]	32%	31%	30%	32%
Maize	**6**[2]		31%	30%	29%	31%
Rat	**13**	**13**		96%	77%	90%
Mouse	**14**	**13**	**0**		77%	90%
Pig	**14**	**14**	**1**	**1**		
Human	**14**	**13**	**1**	**1**	**2**	

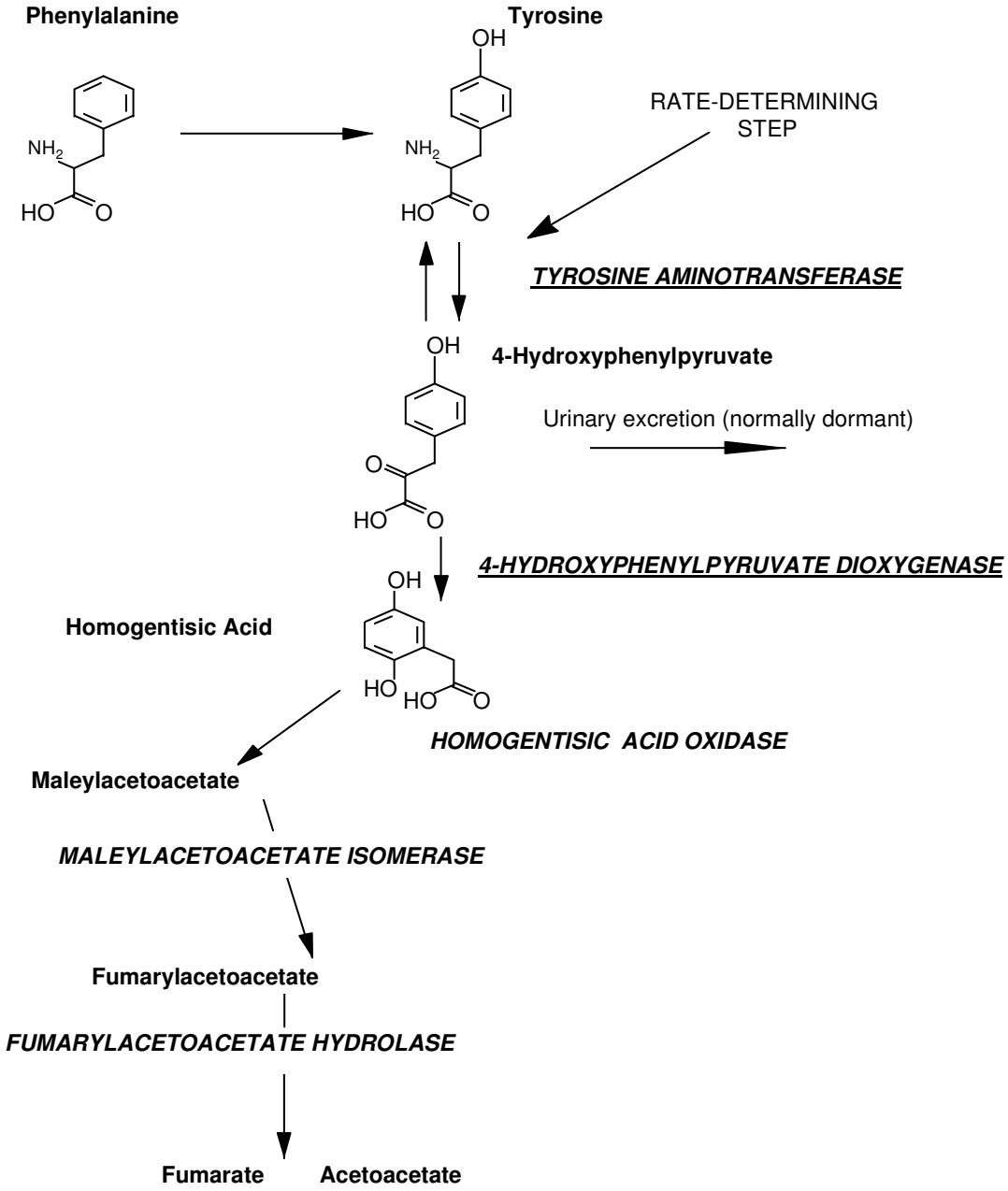
[1]% numbers are the overall % sequence similarity.
[2]Bold numbers are the number of differing active site residues (out of 52 total). From (Yang, et al., 2004)

Source: Syngenta (2014)

It can be concluded that mesotrione will inhibit HPPD in both plants and animals, and this is supported by direct measurements of hepatic and renal enzyme activity in rats and mice, which confirm that mesotrione inhibits HPPD in both species and that this inhibition is reversible (Lock et al., 1994).

HPPD is the second enzyme in the catabolic cascade of tyrosine (Fig. A-1).

Fig. A-1. Catabolic pathway of tyrosine

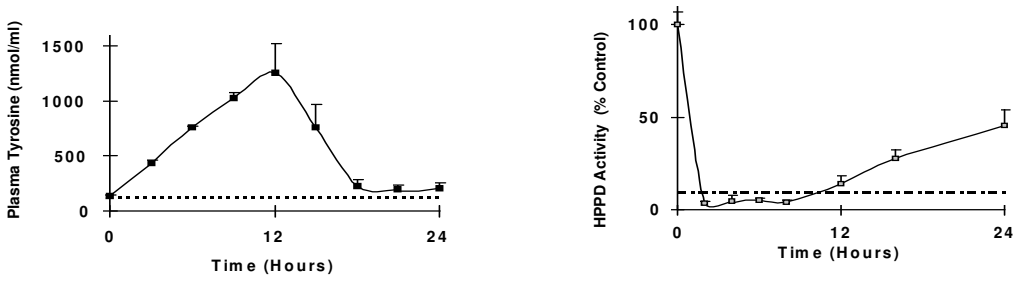


Source: Syngenta (2014)

Increase in plasma tyrosine concentration

The consequence of HPPD inhibition is a dose- and species-dependent elevation in plasma tyrosine levels. If enzyme binding is reversible (as is the case with mesotrione), enzyme activity will recover and plasma tyrosine concentrations will fall once exposure to mesotrione ceases (Brammer, 1995). After a single dose of mesotrione of 2 mg/kg bw administered by gavage to rats, enzyme activity starts to recover after 8 hours (Fig. A-2).

Fig. A-2. The 24-hour plasma tyrosine concentration and HPPD activity in male rats after a 2 mg/kg bw single oral gavage dose of mesotrione



Note 1: dotted line for plasma tyrosine = the mean value in control rats.
Note 2: broken line for HPPD activity = the limit of quantification of the assay used.
Values on each graph represent the mean plus standard deviation, where $n = 5$.

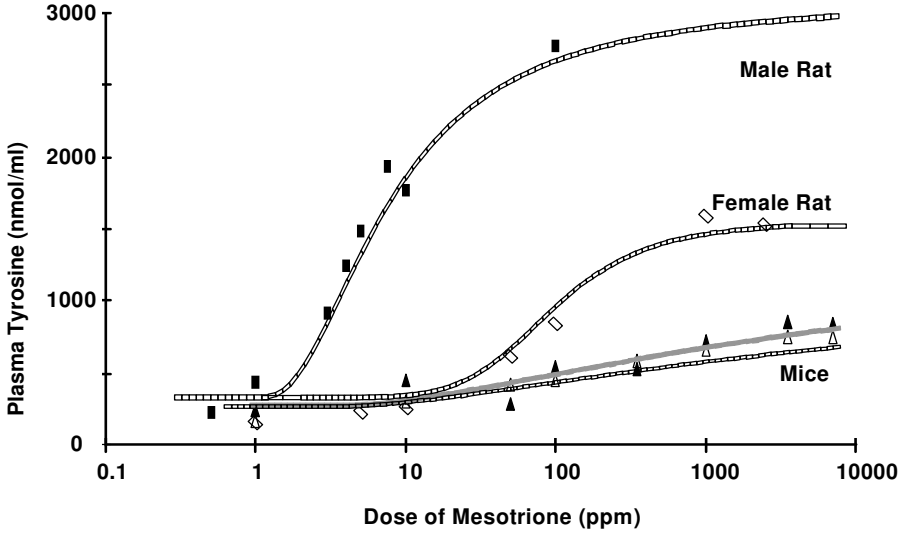
Source: Syngenta (2014)

Clearance of excess tyrosine

Inhibition of HPPD leads to a buildup of its substrate HPPA, which is found in urine (Ellis et al., 1995). The formation of HPPA from tyrosine by TAT is reversible, and a buildup of HPPA results in an elevation of tyrosine levels in the plasma (tyrosinaemia). TAT, the first enzyme in the catabolic pathway, is the limiting and controlling enzyme of tyrosine catabolism. HPPD normally operates at a fraction of its maximum velocity (Lock et al., 1996), and tyrosine concentration is a function of the rate of formation/absorption of tyrosine, the activity of TAT and the efficiency of HPPA elimination by the kidney.

As detailed in Odum (1997), the innate hepatic TAT activity is higher in the mouse, and a sex difference in the rat results in TAT activity being higher in the female rat than in the male rat. The difference in TAT activity results in species and sex differences in tyrosine accumulation (Fig. A-3).

Fig. A-3. Mesotrione-induced tyrosinaemia: dose–response relationship in male and female rats and mice



Source: Syngenta (2014)

Tyrosine-related spectrum of toxicological effects

In standard regulatory studies, a range of toxicities has been seen in rats. Some of these toxicities are seen in mice, but only at very high dose levels (Table A-2).

Table A-2. Toxicities seen in the rat and mouse following mesotrione administration

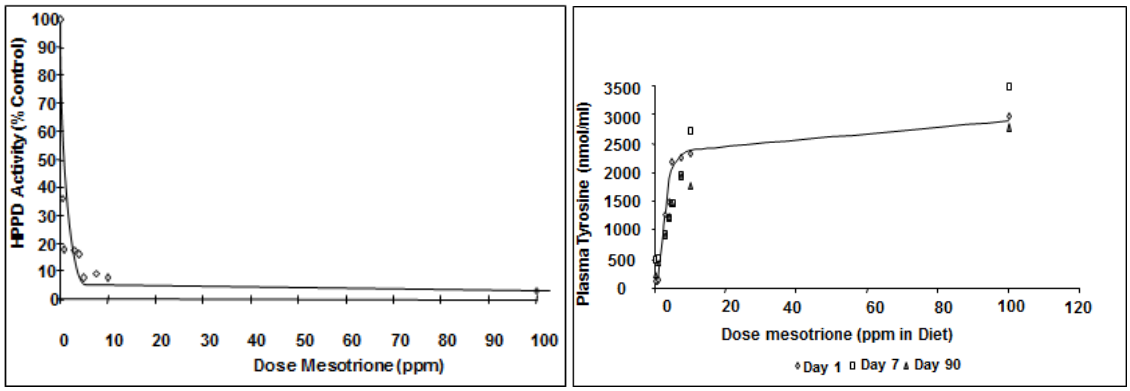
	Presence/absence	
Effect	**Rat**	**Mouse**
Corneal opacity	+	–
Thyroid proliferation	+	–
Sciatic demyelination	+	–
Glomerulonephropathy	+	–
Liver weight increase	+	+/–
Kidney weight increase	+	+/–
Body weight decrease	+	+/–
Reproductive effects		
Litter effects (reduced survival)	+	–
Bilateral hydronephrosis	+	–
Minor modulation in the rate of normal ossification	+	–

Source: Syngenta (2014)

Dose–response relationships

The relationship of dose of mesotrione administered in the diet to the resultant concentration of tyrosine in the plasma is illustrated in Fig. A-3. This correlates with the inhibition of HPPD, as illustrated in Fig. A-4.

Fig. A-4. HPPD inhibition (a) and plasma tyrosine concentration (b) in male rats – 90 day administration



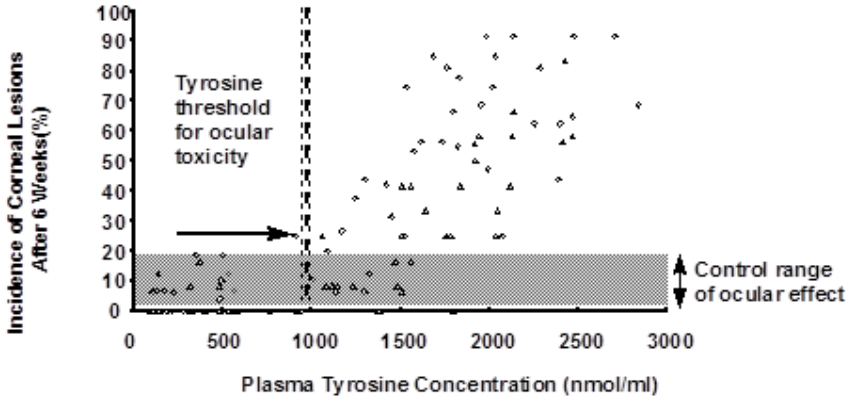
Note: Data taken from studies conducted according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 408

Source: Syngenta (2014)

Tyrosine dose–response relationships: direct association between plasma tyrosine concentration and toxicity

It has been shown (Rich, Beard & Burns, 1973; Burns, Gipson & Murray, 1976; Robinson, 1995) that feeding rats on low-protein/high-tyrosine diets causes corneal opacity identical to that seen in rats administered mesotrione in diets for at least 2 weeks. Data taken from a large series of studies in which groups of rats were dosed for 6 weeks with different triketone HPPD inhibitors showed that there was a linear relationship between plasma tyrosine concentration and ocular toxicity. This study also demonstrated that there was a threshold plasma concentration (approximately 1000 nmol/mL) below which tyrosine-induced corneal lesions do not occur (Fig. A-5).

Fig. A-5. Relationship of plasma tyrosine concentration to corneal lesions in rats



Source: Syngenta (2014)

The dose–response relationship between tyrosine and all adverse effects seen in subchronic and chronic studies in rats has been considered in the non-guideline short-term toxicity studies. In summary, all adverse effects described in the systemic toxicity studies in the rat correlate closely with plasma tyrosine concentration, providing good but indirect evidence that tyrosine, rather than mesotrione itself, is the causal agent of these toxicities.

Strength, consistency and specificity of association

More direct evidence supporting the correlation of tyrosine concentration with toxicological effect is available from exacerbation studies and by examining the species differences in the toxicity of mesotrione.

Tyrosine exacerbation studies

Studies in which HPPD is inhibited by mesotrione and the resulting tyrosinaemia is exacerbated by adding excess tyrosine to the diet have been conducted in pregnant rats to evaluate the effect of tyrosine on pregnancy and in rabbits to investigate the effect of tyrosine on fetal ossification. These studies are discussed in Williams (2000) and Moxon (2000). They show a consistency in the direct role of tyrosine as the cause of developmental and reproductive effects in the rat and rabbit and strengthen the evidence that excessive plasma tyrosine is responsible for the toxicity observed.

Species comparison

The spectrum of toxicities seen in rats following administration of mesotrione is different from that seen in mice administered similar doses and/or doses orders of magnitude higher than those administered to rats. The difference is concluded to be entirely attributable to the significantly higher plasma tyrosine concentrations seen in rats (Table A-3).

Table A-3. Toxicities and plasma tyrosine concentrations seen in the rat and mouse following mesotrione administration

	Rat			Mouse		
Effect	**Presence/ absence**	**Plasma tyrosine[a] (nmol/mL)**	**Mesotrione dose (mg/kg bw per day)**	**Presence/ absence**	**Plasma tyrosine[a] (nmol/mL)**	**Mesotrione dose (mg/kg bw per day)**
Corneal opacity	+	> 1 000	0.16	–	~800	> 1 000
Thyroid proliferation	+	> 1 000	< 0.48	–	~800	> 1 000
Sciatic demyelination	+	> 1 000	< 0.48	–	~800	> 1 000
Glomerulonephropathy	+	> 1 000	< 0.48	–	~800	> 1 000
Liver weight increase	+	~800–1 000	< 0.48	+/–	~800	> 1 000
Kidney weight increase	+	~800–1 000	< 0.48	+/–	~800	> 1 000
Body weight decrease	+	~800–1 000	< 0.48	+/–	~800	> 1 000
Reproductive effects						
Litter effects	+	~800–1 000	1.2	–	~800	> 1 000
Bilateral hydronephrosis	+	~800–1 000	0.3	–	~800	> 1 000
Ossification effects	+	~800–1 000	< 100	–	~800	> 600

[a] Measured or extrapolated from research data.

Source: Syngenta (2014)

Temporal associations and reversibility

The time dependency of the relationship between elevated plasma tyrosine and genesis of an adverse biological event is demonstrated by the data on ocular opacity. A sustained plasma tyrosine elevation in excess of a threshold of approximately 1000 nmol/mL plasma needs to be maintained for 6 weeks to reliably induce ocular change. Most of the pathological findings associated with mesotrione exposure, such as sciatic nerve demyelination, thyroid proliferation and degenerative kidney change, are seen only following exposures longer than 1 year. Furthermore, effects noted in the rat after mesotrione dosing for 90 days are reversible within 4 weeks after cessation of dosing. This reversal coincides with clearance of mesotrione, cessation of HPPD inhibition and return of tyrosine plasma concentrations to control group levels (see Brammer, 1997; Tinston, 1997).

It can be concluded that all early effects occurring with repeated doses of mesotrione are reversible and that all chronic effects are seen only when severe tyrosinaemia is sustained for longer than 1 year (Table A-4).

Life stage sensitivity

Data are available to demonstrate that neonatal rats and mice are not more sensitive than adults to the inhibition of HPPD. Hepatic TAT activity was measured in untreated rats and mice from day 1 postpartum to 6 weeks of age. TAT expression at birth was as high as or higher than adult levels. However, in the case of rats (but not mice) between birth and puberty, the TAT levels of male rats fell, whereas those of the females remained constant (Fig. A-6).

Despite this consistency in TAT expression, the plasma concentration of tyrosine in control mice is higher in pups than in adults (Fig. A-7).

Nevertheless, it has also been shown that elevation of plasma tyrosine, as a consequence of exposure to mesotrione, is lower in mouse pups than in the maternal animal (Fig. A-8).

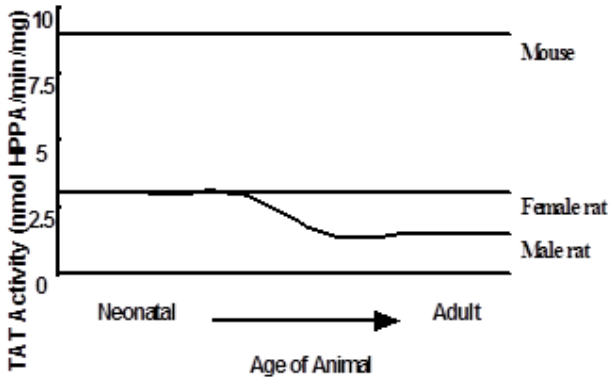
Table A-4. Reversibility of tyrosine-induced toxicity in the rat: values at termination and following a 4-week recovery period in a 90-day study conducted according to OECD Test Guideline 409

	5 ppm		100 ppm		2 500 ppm	
	Week 0	**Week 4**	**Week 0**	**Week 4**	**Week 0**	**Week 4**
HPPD activity (% of control)	11	55	3.5	62	3.8	N/D
Plasma tyrosine concentration (% of control)	828	101	1382	106	1039	374
TAT activity (% of control)	114	104	119	102	166	N/D
Body weight (% difference from control)	−3	0	−2.5	+2	−9	−8
Liver weight/body weight (% of control)	110	101	113	107	120	113
Kidney weight/body weight (% of control)	112	100	112	99	110.5	110
Ocular effects						
NAD/total examined	67/80	15/16	24/80	8/16	28/76	6/16
Opacity	10	0	56	0	46	0
Vascularization	2	0	44	0	41	0
Ghost vessels	0	0	0	0	0	10

HPPD: 4-hydroxyphenylpyruvate dioxygenase; NAD: no abnormality detected; N/D: not determined; OECD: Organisation for Economic Co-operation and Development; TAT: tyrosine aminotransferase

Key: Week 0 = week 13/14 of dosing (90 days)

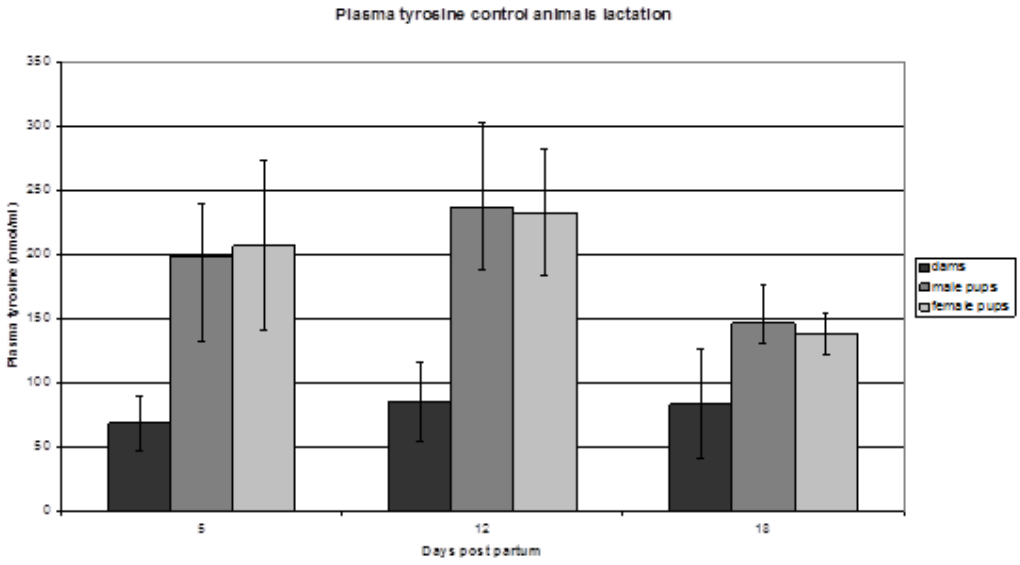
Fig. A-6. Expression of TAT in rats and mice with age



Source: Syngenta (2014)

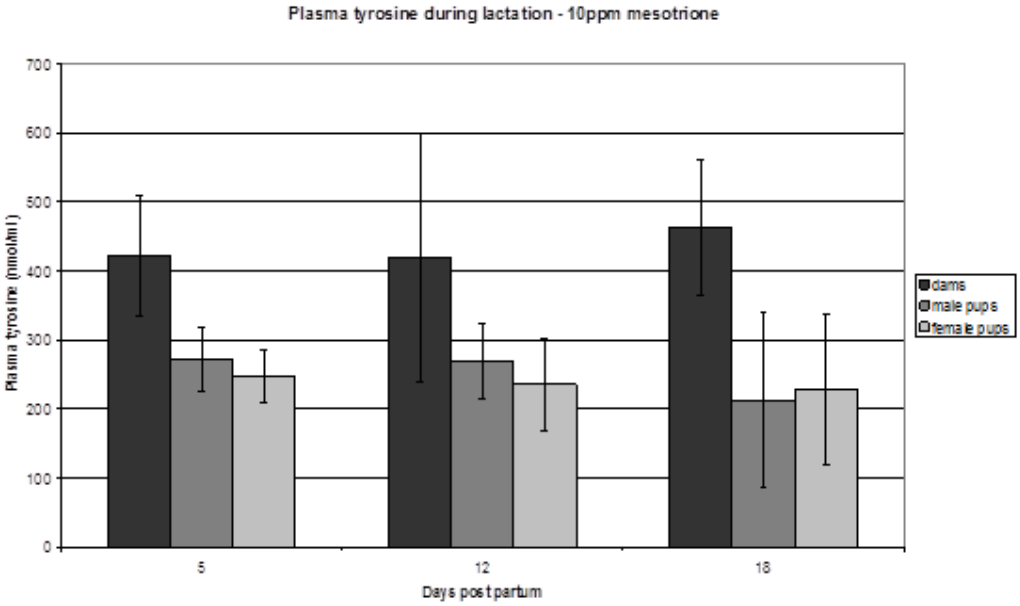
From these data, it can be concluded that plasma tyrosine concentrations in control pups are higher than those in adults. The addition of 10 ppm mesotrione to diet results in a significant elevation of plasma tyrosine in adults, although average levels do not exceed 500 nmol/mL. In contrast, plasma tyrosine levels in pups from the 10 ppm mesotrione group are not significantly elevated above age-matched control levels during lactation and remain less than 500 nmol/mL. These data demonstrate that there is no evidence for increased sensitivity of neonatal animals to the effects of mesotrione.

Fig. A-7. Plasma tyrosine concentration in control animals during lactation



Source: Syngenta (2014)

Fig. A-8. Plasma tyrosine concentration in animals given 10 ppm mesotrione in diet during lactation



Source: Syngenta (2014)

Biological plausibility and coherence of the database

A remarkable consistency is observed in the incidence of plasma tyrosine concentrations in excess of 1000 nmol/mL and the occurrence of adverse effects (ocular change, liver and kidney weight increase, reduced body weight and exacerbation of a range of spontaneous pathologies).

In the assessment of early life stage toxicity in the developmental and reproductive database, the same marked association between the biological end-points of minor changes in ossification and reduced pup survival and elevated plasma tyrosine levels has been demonstrated.

There is also consistency between the systemic and reproductive toxicity databases, where the same elevated liver and kidney weights, reduced body weights and ocular changes are again observed in the presence of elevations in plasma tyrosine levels.

The key events in the animal MOA are summarized in Table A-5.

Table A-5. Key events in the animal MOA

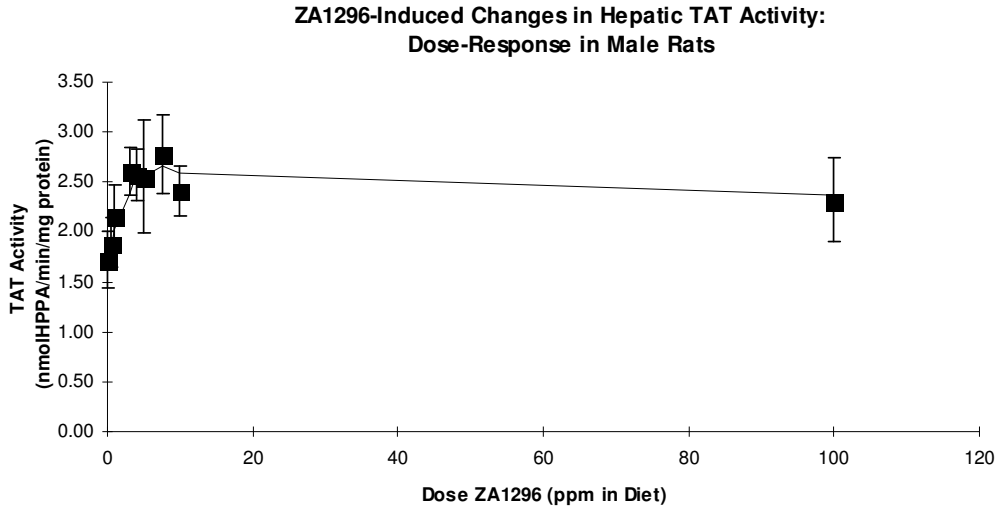
Key event	Evidence	References
Inhibition of HPPD	• YES: This is the established herbicidal MOA in plants • Has been shown to be the MOA for HPDD inhibitors in mammals • HPPD inhibitors are structurally diverse, but have a common substructure, which binds tightly to a single, common active site in both plant and mammalian HPPD • HPPD active site sequence ➢ similar across plants and animals ➢ highly conserved across mammalian species	Lock et al. (1994); Lee et al. (1997)
Increase in systemic tyrosine concentrations	• YES: Increases in plasma tyrosine are mesotrione dose dependent in rats, mice and humans • Degree of tyrosinaemia is species specific and dependent upon the innate activity of TAT, the rate-limiting enzyme in the tyrosine catabolic pathway	Nixon (2001)
Clearance of excess tyrosine via TAT	• YES: TAT is the first enzyme in the catabolic pathway for tyrosine. If the second enzyme (HPPD) is inhibited, excess tyrosine is cleared as phenolic acids in the urine. Rate of clearance depends on inherent activity of TAT, which is species dependent – higher in the mouse than in the rat (mouse > 2× female rat/4× male rat)	Nixon (2001)
Tyrosine-related spectrum of toxicological effects	• YES: Mesotrione administration results in effects on body weight, liver, kidney, eyes, thyroid in subchronic/chronic rat studies and on reproduction/development in the rat. These effects are attributable to severe tyrosinaemia based on: ➢ direct causation of effect by tyrosine alone (ocular opacity) ➢ correlation of effect with tyrosine levels – chronic effect on body weight, liver and kidney, sciatic nerve and thyroid ➢ exacerbation – effect of mesotrione and excess dietary tyrosine on fetal ossification and reproduction	Rich, Beard & Burns (1973); Burns, Gipson & Murray (1976)

HPPD: 4-hydroxyphenylpyruvate dioxygenase; MOA: mode of action; TAT: tyrosine aminotransferase

Alternative MOAs

Given the strength and consistency of the correlation of tyrosine levels to various toxicological outcomes, the obvious species-dependent generation of high plasma tyrosine levels and the clear dose–response relationship for tyrosine rather than mesotrione, it is unlikely that there are alternative MOAs. No other MOAs have been identified for mesotrione that are able to unite the range of biological effects observed. Mesotrione does not inhibit TAT, the first enzyme in the tyrosine catabolic cascade (Fig. A-9).

Fig. A-9. Mesotrione-induced changes in hepatic TAT activity – male rats



Source: Syngenta (2014)

Nuclear magnetic resonance analysis of urine from rats dosed with mesotrione shows excess phenolic acids (Lock et al., 1996), which is consistent with the inhibition of HPPD.

There was no evidence, from urinary analysis, of excess levels of tyrosine itself, tyramine or *N*-acetyltyrosine, which would have indicated TAT inhibition, or of fumarylacetoacetate, maleylacetoacetate or homogentisate, which would have indicated an inhibition of fumarylacetoacetate hydroxylase, maleylacetoacetate isomerase or homogentisic acid oxidase. In addition, mesotrione does not inhibit tyrosine hydroxylase, a key enzyme in the anabolic pathway of tyrosine. Therefore, mesotrione has been shown to inhibit a single enzyme (HPPD) in the catabolic pathway of tyrosine and to not affect the key enzyme in the anabolic pathway.

There are some other MOAs that are known to operate with other HPPD inhibitors, such as hepatic P450 induction. The lack of significant P450 induction by mesotrione was confirmed by the modest increase in liver weight in chronic studies, the lack of pathological changes in the liver and a short-term study (Odum, 1997) showing that the relevant enzymes were not induced.

Conclusions: Assessment of postulated MOA in animals and statement of confidence

Overall, there is a high level of confidence in the postulated MOA that tyrosine elevation leads to a range of biological effects, which are consistent with those seen predominantly in rats following mesotrione exposure. This confidence is based on evidence for direct causation between plasma tyrosine levels and ocular changes, where ocular tyrosine concentrations and effects in the eye are highly correlated. Furthermore, there is a large and convincing database showing a consistent positive correlation between all the biological end-points described following mesotrione treatment and elevations in plasma tyrosine concentration. Additionally, tyrosine exacerbation studies have established a firm link between the severity of developmental and reproductive changes and the degree of elevation of plasma tyrosine concentration.

It is concluded that the weight of evidence is sufficient to establish an MOA in animals.

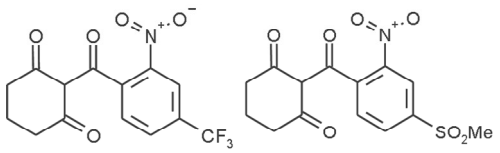
Are the key events in the animal MOA plausible in humans?

HPPD inhibition and increase in tyrosine

Humans have an HPPD that is similar to both the rat and mouse HPPD; in particular, the amino acid sequence homology in the active site is nearly identical, differing by only one amino acid

residue. NTBC (also known as nitisinone and Orfadin®) is an HPPD inhibitor (Lock et al., 1994) that is structurally similar to mesotrione (Fig. A-10) and is used therapeutically to treat humans with tyrosinaemia type 1 (an autosomal recessive fumarylacetoacetate hydrolase deficiency).

Fig. A-10. Chemical structures of NTBC and mesotrione



Nitisinone (NTBC) Mesotrione

Source: Syngenta (2014)

Human data are available from volunteer studies, clinical trials and over 2000 patient years of experience that demonstrate that HPPD inhibitors (NTBC and mesotrione) cause an increase in plasma tyrosine concentration (Lindstedt et al., 1992; Hall et al., 2001). Therefore, based on HPPD enzyme similarities between humans and rodents and based on studies with HPPD inhibitors, it is considered that mesotrione is able to inhibit human HPPD and can increase plasma tyrosine concentrations.

Clearance of tyrosine via TAT

The level of TAT activity in humans is similar to that of male mice, and humans are thus able to efficiently catabolize tyrosine (Table A-6).

Table A-6. Comparison of innate hepatic TAT activity – rats, mice and humans

	Hepatic TAT activity (nmol HPPA/min/mg protein)		
	Rat	**Mouse**	**Humans[a]**
Males	1.7 ± 0.2	7.8 ± 1.5	7.17 ± 1.17
Females	3.3 ± 0.5	10.5 ± 1.9	

HPPA: 4-hydroxyphenylpyruvate; TAT: tyrosine aminotransferase

[a] From Henderson et al. (1981).

As is the case for mice and rats, TAT is fully active 24 hours after birth (Kretchmer, 1959).

Tyrosine-related spectrum of toxicological effects

There are limited reliable data on toxicity in humans occurring as a consequence of elevated plasma tyrosine concentrations. In patients treated with NTBC, it is reported that eye disorders, including conjunctivitis, photophobia, eye pain, keratitis and corneal lesions, have been noted, some of which were transient and/or reappeared (USFDA, 2013), although none occurred in adults treated for alkaptonuria (Sunwannarat et al., 2005). Although alkaptonuria patients were recorded with plasma tyrosine concentrations that periodically reached 800 nmol/mL, none showed treatment-related ocular effects. However, there are no data from patients with plasma tyrosine concentrations that significantly exceed these concentrations for prolonged periods.

Nonetheless, there is no evidence to suggest that humans would react differently from rats to high, sustained levels of tyrosine. For NTBC, it is recognized that humans could exhibit adverse effects should tyrosine levels reach toxicologically relevant concentrations, and the USFDA (2013) therefore recommended that plasma tyrosine levels should be kept below 500 µmol/L in order to

avoid toxic effects (i.e. corneal lesions and hyperkeratotic lesions and neurological symptoms) caused by high plasma tyrosine levels.

In summary, the key events seen predominantly in rats are plausible in humans, although there are significant species differences in the level of plasma tyrosine that can accumulate.

Taking into account kinetic and dynamic factors, is the animal MOA plausible in humans?

The MOA for mesotrione-related adverse effects seen predominantly in rats depends on the sustained elevation of tyrosine. Two factors determine whether or not humans would sustain high plasma tyrosine levels: (1) residence time of the HPPD inhibitor in the body (basic kinetics) and (2) the efficiency of clearance of tyrosine by TAT (Table A-7).

Table A-7. Key events in the animal MOA and human relevance

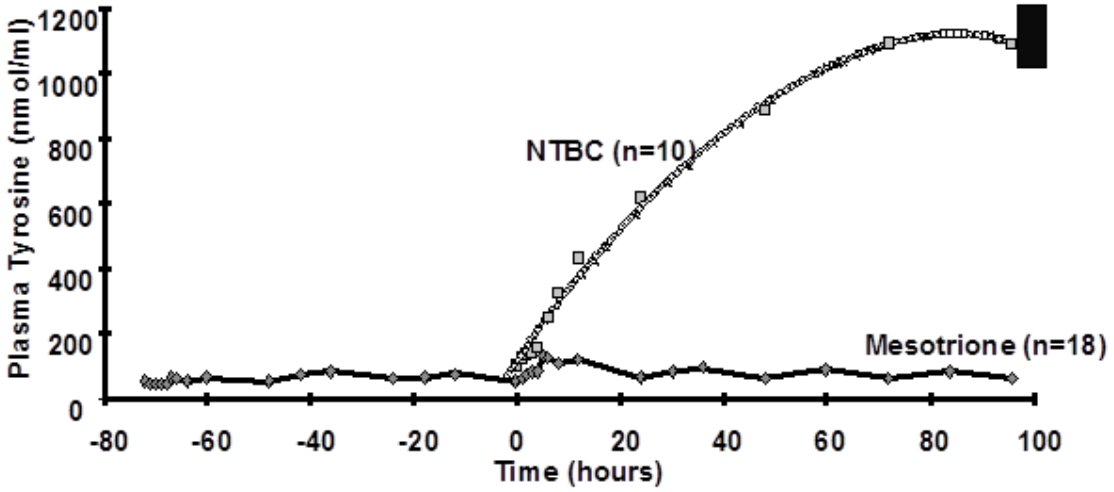
Key event	Evidence in rats	Evidence in mice	Evidence in humans	References
Inhibition of HPPD	• YES: Mesotrione – measured in rats	• YES: Mesotrione – measured in mice	• YES: Indirect data from HT1 patients treated with NTBC	Lindstedt et al. (1992)
Increase in systemic tyrosine concentrations	• YES: Dose-dependent increase in plasma tyrosine concentration	• YES: Dose-dependent increase in plasma tyrosine concentration	• YES: Dose-dependent increase in plasma tyrosine concentration	
Clearance of excess tyrosine via TAT	• YES: Tyrosine cleared slowly • TAT activity (nmol HPPA/min/mg protein) ➢ 1.7 (males) ➢ 3.3 (females) • Maximum plasma tyrosine concentration (nmol/mL) ➢ 2 500–3 000 (males) ➢ 1 500–2 000 (females)	• YES: • TAT activity (nmol HPPA/ min/mg protein) ➢ 7.8 (males) ➢ 10.5 (females) • Maximum plasma tyrosine concentration is 800 nmol/mL	• YES: • TAT activity (nmol HPPA/min/mg protein) ➢ 7.17 • Plasma tyrosine maximum concentration similar to that in mouse when HPPD completely inhibited	Henderson et al. (1981) Hall et al. (2001)
Tyrosine-related spectrum of toxicological effects	• Ocular toxicity evident when plasma tyrosine concentration > 1 000 nmol/mL • Chronic effects and reproductive/ developmental effects at similar plasma tyrosine concentrations	• Minimal effects on liver and kidney weight seen in mouse studies – plasma tyrosine < 1 000 nmol/mL	• No adverse effects seen in human volunteer study with mesotrione or in healthy volunteers with NTBC • NTBC showed minor effects in patients if plasma tyrosine exceeds 800–1 000 nmol/mL • NTBC has no adverse effects in adults treated for alkaptonuria where plasma tyrosine reaches 600–700 nmol/mL	Hall et al. (2001) USFDA (2013) Sunwannarat et al. (2005)

HPPD: 4-hydroxyphenylpyruvate dioxygenase; HT1: tyrosinaemia type 1; MOA: mode of action; NTBC: 2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione; TAT: tyrosine aminotransferase

Kinetics

Experience with NTBC, a potent HPPD inhibitor used as a drug in children for the treatment of tyrosinaemia type 1 (HT1), shows that elevated plasma tyrosine levels can be achieved with this drug. Limited data for mesotrione indicate that comparable doses do not cause significantly elevated tyrosine levels in human volunteer studies (Fig. A-11), although higher doses (4 mg/kg bw) can produce minor transient elevations in plasma tyrosine (Hall et al., 2001).

Fig. A-11. Plasma tyrosine levels in humans after NTBC or mesotrione dosing



NTBC: 1 mg/kg bw; mesotrione: 0.5 mg/kg bw

Source: Syngenta (2014)

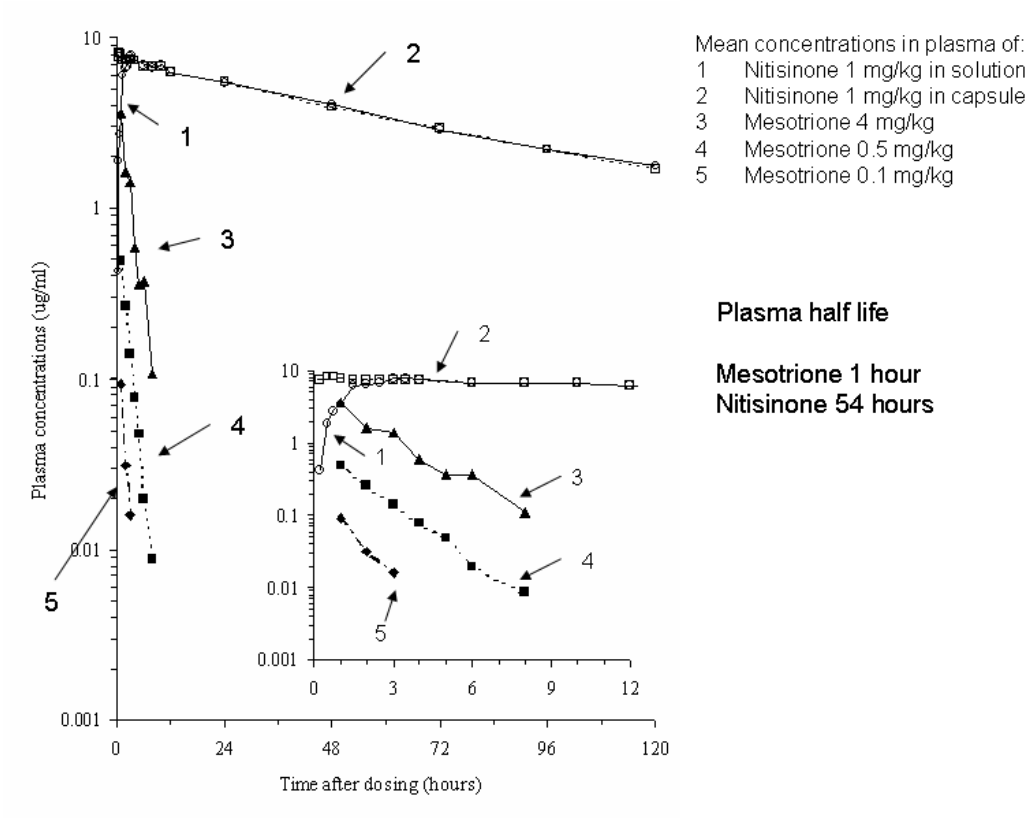
The reason for the difference between NTBC and mesotrione plasma tyrosine concentrations is that NTBC has a plasma half-life of 54 hours compared with mesotrione's half-life of 1 hour (Fig. A-12), resulting in a plasma area under the concentration–time curve that is 400 times greater for NTBC than for mesotrione. The sustained presence of NTBC results in elevated tyrosine levels that plateau at about 1000 nmol/mL.

Therefore, the kinetics of an HPPD inhibitor are important in understanding whether or not the MOA would, in reality, occur in humans. Mesotrione's short half-life would suggest that the MOA would not result in adverse findings in humans unless very high repetitive doses were administered to elevate tyrosine levels and for a sustained period of time.

Clearance of tyrosine

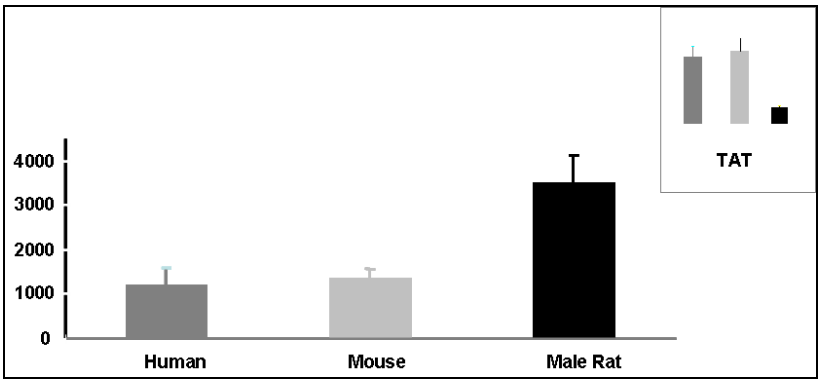
The toxicities attributable to mesotrione have been shown to be significantly different in the two rodent species studied in detail, rats and mice (Table A-2). The metabolic fate of mesotrione has been studied following single and repeated doses in rats and mice, and it has been demonstrated that there are no species differences in the metabolism and excretion of mesotrione that could explain the species differences in toxicity reported (see metabolism studies in sections 1.1 and 1.2 and Gledhill, Jones & Laird, 2001). The differences in toxicity profile are attributable to differences in the steady-state plasma tyrosine concentrations under conditions of complete HPPD inhibition, which in turn have been shown to be dependent upon the animals' innate TAT activity. Human TAT activity is much higher than that of the rat; thus, at equivalent doses of the potent HPPD inhibitor NTBC, plasma tyrosine concentrations in mice and humans are much lower than those seen in the rat (Fig. A-13).

Fig. A-12. Clearance of NTBC or mesotrione from human volunteers



Source: Syngenta (2014)

Fig. A-13. NTBC-induced plasma tyrosine (nmol/mL) elevation in rats, mice and humans



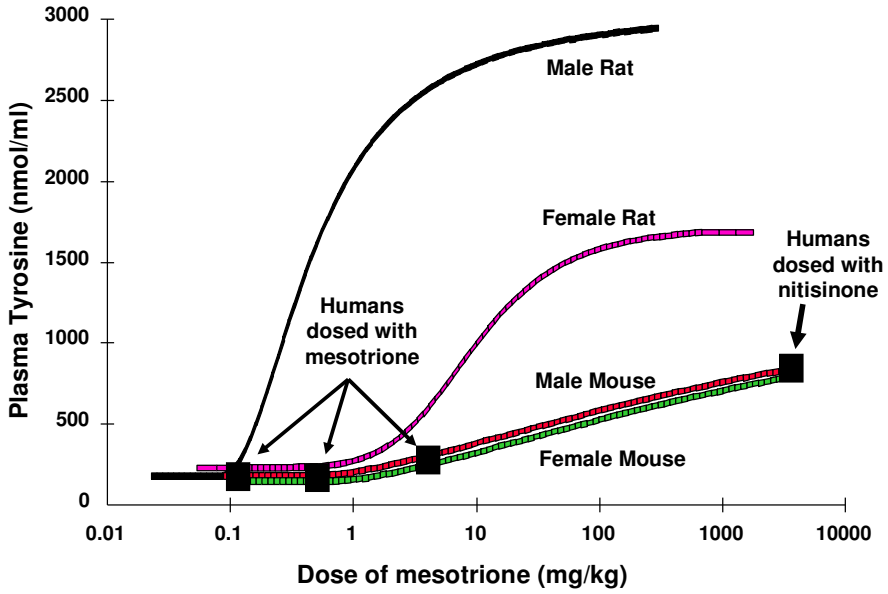
NTBC dose = 1 mg/kg bw
Human data from clinical trial in 10 male volunteers (Hall et al., 2001)
Data for rat from Lock et al. (1996)
Data for mouse from Lock et al. (2000)

Source: Syngenta (2014)

Using values taken from the human volunteer study with mesotrione and the clinical trial with NTBC (Hall et al., 2001) and assuming a 400- to 1000-fold difference in potency of NTBC and mesotrione, it is possible to extrapolate steady-state plasma tyrosine concentrations in humans following mesotrione exposure. These data points have been added to the graph produced for mice and rats and show that humans achieve tyrosine levels similar to those of mice and that these do not

exceed 1000 nmol/mL (the threshold established for induction of adverse effects seen in the rat) (Fig. A-14).

Fig. A-14. Mesotrione-induced tyrosinaemia in mice, rats and humans: relationship between plasma tyrosine concentrations and dose of mesotrione



Source: Syngenta (2014)

On the basis of these data, which clearly indicate that humans will not achieve the high tyrosine levels seen in the rat, the effects of mesotrione in humans would be expected to be the same as those seen in the mouse and to be significantly different from those seen in the rat.

In summary, the kinetic and dynamic characteristics of mesotrione in humans are such that the short half-life (kinetics) and the efficient removal of tyrosine (dynamics) obviate the likelihood of any tyrosine-related effects that are seen in the rat after mesotrione dosing. Therefore, taking into account kinetic and dynamic factors, the animal MOA is plausible in humans, but with the practical certainty that adverse effects will not be observed in humans.

Conclusion and statement of confidence

Based on the available data, the MOA and the key events for mesotrione-related adverse effects have been identified and, on a qualitative basis, are plausible in humans. Given the quantitative factors (kinetics and dynamics) of this MOA (short half-life and the significant differences in TAT activity between humans and rats), humans are unlikely to exhibit the toxicities seen in rats. Nonetheless, mesotrione at some relatively high dose level may raise tyrosine levels in humans, but certainly not to an extent or for a duration that is likely to cause adverse effects.

In addition, the available data lead to the conclusion that for tyrosine-related toxicities, the mouse is the most appropriate model to use for the human risk assessment of mesotrione.

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